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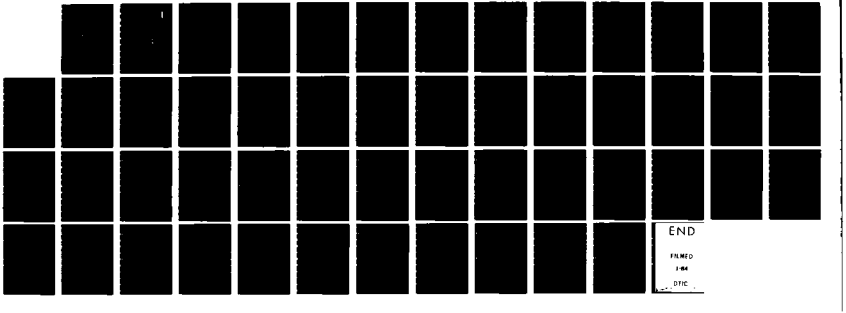

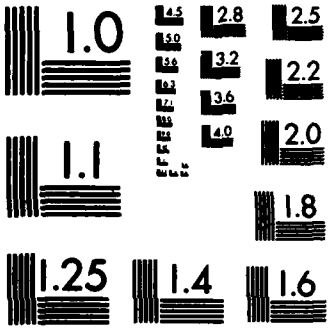

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Final report

AUTHOR

Frank L. Adler, Ph.D.

DATE

9 December 1983

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-83-G-9524

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The Second International Workshop on Immunogenetics and Immunobiology of the Rabbit was held at St. Jude Children's Research Hospital, Memphis, TN, on April 7-9, 1983. It was attended by approximately 90 immunologists from the USA, Canada, France, Belgium, England, Czechoslovakia and Japan. In five half-day sessions poster presentations were followed by workshop discussions led by chairpersons with expertise in the area under discussion. They were joined by two discussants who integrated information obtained from rabbit models into a broader framework. Topics discussed were genetics and structure of immunoglobulins, control of allotype and idiotype expression, lymphocyte differentiation and function, lymphocyte antigens, and rabbit models for allergy and infectious diseases. A summary report of the proceedings has been accepted for publication by "Cellular Immunology." Copies of abstracts of presentations and summary reports for each of the five sessions have been distributed to the registered participants. A closing session dealt with the problem of sharing of resources, such as typing sera, monoclonal antibodies and rabbits defined with respect to immunoglobulin allotypes and major histocompatibility antigens. An international committee was established (F.L. Adler, Chairman) which is charged with responsibility to facilitate sharing of resources and preservation of genetically defined rabbits.

TERMINAL REPORT

SECOND INTERNATIONAL WORKSHOP ON IMMUNOGENETICS AND IMMUNOBIOLOGY OF THE RABBIT

An International Workshop on Immunogenetics and Immunobiology of the Rabbit was held at St. Jude Children's Research Hospital, Memphis, Tennessee, on April 7 to 9, 1983. Members of the Organizing Committee were:

1. Louise T. Adler, Ph.D., Associate Member, Division of Immunology, St. Jude Children's Research Hospital, Memphis, TN (Chairperson).

2. Frank L. Adler, Ph.D., Member and Chairman, Division of Immunology, St. Jude Children's Research Hospital, Memphis, TN.

3. William J. Mandy, Ph.D., Professor and Chairman, Department of Microbiology, The University of Texas at Austin, Austin, TX.

4. L. Scott Rodkey, Ph.D., Associate Professor, Department of Pathology, University of Texas Medical School, Houston, TX.

5. Dennis W. Metzger, Ph.D., Assistant Member, Division of Immunology, St. Jude Children's Research Hospital, Memphis, TN.

The primary purpose of this workshop was to convene investigators who use rabbits as experimental models for the study of immunoglobulin genetics, structure, and regulation as well as for the study of infectious and allergic diseases. A number of other individuals, chosen specifically for their ability to provide perspective and to relate data obtained with rabbit models into a broader context of current immunological research, were also invited (program enclosed).

The workshop was attended by approximately 90 immunologists (participant list enclosed), representing institutions in the U.S., Canada, France, Belgium, England, Czechoslovakia, and Japan. Attendance by young investigators (graduate students, postdoctoral fellows, and junior faculty) working with rabbit models was encouraged by offering partial travel support to qualified individuals in these categories.

The meeting was organized into 5 half-day sessions. The format for each consisted of poster presentations followed by discussion of the data in a workshop led by 2 Chairpersons chosen for their expertise in the area under consideration. These individuals were joined by 2 discussants who contributed towards integrating this new material into a broader framework.

Abstracts were solicited 4 months prior to the meeting date under the general headings of Genetics and Structure of Rabbit Immunoglobulin, Control of Allotype and Idiotype Expression, Lymphocyte Differentiation and Function, Rabbit Lymphocyte Antigens, and Rabbit Models for Allergy and Infectious Diseases. In all, 62 abstracts were accepted and were made available to the participants at the beginning of the meeting (see enclosure). The format employed, poster

sessions followed by organized discussion, resulted in a lively exchange of information and opinions. The Chairpersons and Discussants succeeded in carrying out the intentions of the Organizing Committee by eliciting clarifications and further information in the workshops, rather than a reiteration of data already presented on posters. Very few slides were shown. Evaluation of the meeting by participants resulted in almost uniformly enthusiastic approval of the format and arrangements. The consensus of the group was that a similar meeting should take place every 2 to 3 years. The first workshop on this subject was held in May, 1978, in Bethesda, under the sponsorship of NIAID.

The current workshop achieved its objectives through a clear demonstration of cohesiveness, across international boundaries, of investigators and their willingness to share ideas and resources. It became quite apparent that the rabbit which has in the past provided many original insights into immunoglobulin structure, allotypes, idiotypes and their functions, has more to offer in the future. Rapid progress has been made in the application of hybridoma technology toward the assembly of reagents useful in the classification of lymphoid cells; the advent of rabbit/rabbit hybridomas appears imminent, and cDNA probes have been prepared and used for studies on H and L chains with particular emphasis on information relevant to allotypic markers. Recent findings of the inappropriate expression of allotypes not believed to be present in the genotype have emphasized the need for information on the DNA level to obtain a better understanding of the genetics and controls governing allotype expression.

Recent advances in the establishment of lymphoid cell chimerism using donor and recipient rabbits which are identical at the major histocompatiblity locus (RLA) but otherwise unrelated, have provided a new and practical model appropriate to research on bone marrow transplantation in man, another outbred species. For the study of allergic and infectious diseases the rabbit provides a laboratory model for symphilis as well as excellent opportunities for studies on the role of bacterial adherence to the intestinal epithelium in diseases caused by pathogenic E. coli and on the phenomenon of latency in herpes virus infections. Furthermore, the rabbit continues to be the model of choice in studies on immune responses following ingestion of antigens and shows promise in studies on immune complex arthritis and IgE-mediated asthma.

Reports on the scientific content of this workshop will take 2 forms: (1) a Meeting Report (in preparation) will be published in Cellular Immunology and (2) summaries of individual sessions submitted by the Chairmen are being compiled and copies will be sent to all registrants.

The meeting closed with discussion of a report by an ad hoc Committee on Rabbit Resources. It was concluded that continuation of research at the present or accelerating rate is dependent upon a continued supply of pedigreed rabbits, currently not available from commercial sources. Investigators needing such animals have in the past relied upon a system of mutual international cooperation. The feasibility of this system is now seriously threatened by increased costs and budget cutbacks. Some entire colonies have already been liquidated. In view of this alarming situation, the participants of the

workshop authorized formation of an International Committee on Rabbit Resources to study the problem and report to the next workshop, tentatively scheduled as a satellite meeting of the Sixth International Congress of Immunology, to be held in Toronto in 1986. Frank Adler, one of this workshop's organizers, was charged with the formation and organization of this committee.

Louise T. Adler

Louise T. Adler, Ph.D.
Associate Member
Division of Immunology

MEETING REPORT

SECOND INTERNATIONAL WORKSHOP ON IMMUNOGENETICS AND IMMUNOBIOLOGY OF THE RABBIT[1]

Louise T. Adler,[2] Frank L. Adler,[2] William J. Mandy,[3]
L. Scott Rodkey,[4] and Dennis W. Metzger[2]

[1]This meeting was supported by grants from the National Institutes of Health (AI-19097), the National Science Foundation (PCM-8216802), and the U.S. Army Medical Research and Development Command (DAMD 17-83-G-9524) as well as by gifts from Accurate Chemical and Scientific Corporation and Cumberland View Farms.

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The Second International Workshop on Immunogenetics and Immunobiology of the Rabbit was held at St. Jude Children's Research Hospital in Memphis, Tennessee, on April 7-9, 1983. This conference was attended by 90 participants representing laboratories in the U.S., Canada, France, Belgium, Great Britain and Japan, who met to discuss recent advances in studies ranging from molecular genetics to transplantation and disease in which rabbits were used as the experimental model.

The five sessions dealt in depth with the genetics and structure of rabbit immunoglobulins, the control of allotype and idiotype expression, rabbit lymphocyte antigens, lymphocyte differentiation and function, and rabbit models for studies on allergy and infectious diseases. Data were presented in poster sessions, followed by workshop discussion periods to which invited panelists with related expertise acquired by the use of animal models other than the rabbit were invited. This format proved useful in delineating the role of the rabbit in present and future immunological research.

In the first session data were presented on the organization and regulated expression of rabbit light chain genes. Several laboratories have now constructed and sequenced cDNA clones for κ chains of the b4, b5 and b9 allotypes. Short probes that distinguish b5 from b9 mRNAs have also recently been constructed. Information on J_K genes and the associated C_K gene encoding the b4 light chain led to discussion of "hidden" information for normally unexpressed (latent) allotypes. While Southern blotting with J_K and C_K probes from nominally b4 rabbits reveals several bands, the data at this point do not support the concept that the genetic information for all of the kappa allotypes is present in every rabbit. Thus, the genetic basis for the reported expression of latent allotypes still remains to be established, but rapidly developing technical advances should provide answers soon. Concerning

the organization of the genome, it appears that the rabbit has, unlike man or mouse, more than one segment encoding J_K regions. Five exons encoding J-regions associated with the b4 C_K gene have been found, but only one appears to be functional. Other data suggest that separate, cross-hybridizing C_K genes exist; their functional status remains to be determined. Thus, the organization of the rabbit's J_K and C_K genes may be analogous to the duplicated regions of Jλ and Cλ of the mouse.

Base sequences of cDNAs for μ and γ heavy chains as well as DNA sequences for genomic Cγ, a1, a2, and a-negative V_H genes were also reported. A 60 bp probe from V_H a2 was shown to be allotype-specific in hybridization to mRNA from a1, a2, and a3 rabbits. These studies, together with the application of monoclonal anti-allotype reagents to serological studies on domestic and wild rabbits, emphasize the complexity of the polymorphism of rabbit immunoglobulin. Finally, the need for stable rabbit cell lines was widely recognized and some progress was noted.

Spirited discussion was evoked by the reports on the high incidence of cross-reactive idiotypes among rabbit antibodies against allotypic determinants on rabbit immunoglobulins. The sharing of such idiotopes by xenogeneic antibodies was taken to argue against the concept of highly conserved relevant rabbit genes. The alternate concept, that of an "internal image," received much discussion, with emphasis on experimental strategies that are required to distinguish between molecules bearing such epitopes and molecules that express anti-idiotypic paratopes. With regard to regulation of Ig expression, it was reported that Basilea rabbits, which produce mostly Ig molecules bearing λ light chains, have pre-B cells with b9 or b9-like chains in their cytoplasm. The apparently aborted differentiation to cells secreting Ig with the b9 κ light chain was discussed in terms of experimentally induced

chronic allotype suppression, a subject further considered in the light of experiments with chimeric rabbits. There it was noted that cells from a suppressed donor, transferred to newborn histocompatible recipients, remained suppressed. However, transfer of such cells to newborn recipients deliberately mismatched to facilitate graft vs. host reactions, resulted in release from allotype suppression, possibly due to intense allogeneic stimulation. Preliminary data from functional studies on donor-derived B lymphocytes in chimeric rabbits suggest that the rabbit may be a useful model for the study of immunodeficiencies in human bone marrow recipients.

The detection of rabbit lymphocyte subsets is being greatly aided by the use of monoclonal antibodies, as indicated by the number of poster presentations on this subject. It was noted that a dominant epitope on rabbit T lymphocytes is shared with most or all PMN cells of the rabbit, as revealed by the specificities of three hybridomas made in separate laboratories. A listing of available hybridomas was prepared and distributed to the participants. Also discussed at length was the polyclonal activation of rabbit B cells, directly through anti-Ig reagents or indirectly through T cell activation. Among the polyclonal stimulators discussed was a proteinase-like serum α-macroglobulin and a supernatant factor from cultures of rabbit milk cells. Yet another topic considered was the nature of the rabbit T cell receptor. Studies on solubilized antigen-binding material from T cells of immunized rabbits, using immunoadsorbtion techniques, strongly suggested that the T cell receptor lacks an intact V_H sequence. However, other data suggest the presence of both V_H and L-chain determinants on the antigen-specific receptors of T cells.

The last session of the meeting helped to re-introduce the rabbit as the animal model of choice for studies on syphilis. Less well known are

the suitability of the rabbit for studies in hypersensitivity pneumonitis, IgE-mediated asthma, and immune complex arthritis, as well as the unique opportunities offered by this species for studies on sensitization by the oral route. Additional results presented dealt with the use of rabbit in studies on the adherence of pathogenic E. coli to mucosal cells and also for studies on herpes virus-induced keratitis which hold promise for further work on latent viral infections. A particularly timely report described a fatal systemic immune suppression caused in the rabbit by a DNA pox virus which is a recombinant between Shope fibroma and Shope myxoma viruses.

The meeting reinforced the idea that the rabbit, historically of major significance in studies on immunoglobulin structure and function, continues to offer numerous and often unique opportunities for innovative studies and new insights. In contemporary and future studies the traditional "New Zealand White" needs to be replaced by the pedigreed rabbit, derined with respect to immunoglobulin allotypes, major histocompatibility type, and also with respect to biologically significant deficiencies in enzymes, hormones, or complement components. The continued availability of such animals, none even now available from commercial sources, is seriously threatened. Therefore, a standing Committee on Rabbit Resources was established by unanimous vote of the participants. The committee is to explore and initiate ways and means for the preservation of the required defined animals, to facilitate the sharing of resources, and to seek support for this endeavor. The Third International Workshop has been scheduled for 1986.

APPENDIX A

TO REPORT ON GRANT NO. DAMD17-83-G-9524

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APPENDIX B

2nd INTERNATIONAL WORKSHOP ON IMMUNOGENETICS AND IMMUNOBIOLOGY OF THE RABBIT
ST. JUDE CHILDREN'S RESEARCH HOSPITAL
MEMPHIS, TN
April 7-9, 1983

ORGANIZING COMMITTEE

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TABLE OF CONTENTS

This meeting is supported by grants from the National Institutes of Health, the National Science Foundation, and the U.S. Army Medical Research and Development Command, as well as by gifts from Accurate Chemical and Scientific Corporation and Cumberland View Farms.

Members of the Organizing Committee also wish to express their gratitude to Ms. Chris Winston, Secretary for the Division of Immunology, and Ms. Erlene V. Kirwen, Volunteer Secretary, for their dedicated and invaluable assistance in arranging this meeting.

A. RABBIT IG: GENETICS AND STRUCTURE

A-1

"Structural studies of domestic and wild rabbit immunoglobulin light chain allotype"

by Hammadi AYADI

Institut Jacques Monod, CNRS and Universite Paris VII, Tour 43, Immunologie Moleculaire (43-44), 2 place Jussieu, Paris 5, France.

The constant region kappa light chain locus of the domestic rabbit consists of several genes coding for the b allotypes, defined by Oudin in 1956 (1). Breeding studies suggest that the different b allotypes are products of codominant allelic structural genes. Sequence studies reveal differences of 20 to 35% between the amino acids of the b4, b6 and b9 C_k regions. In addition, a small number of substitutions appear to distinguish constant regions of the same allotype. To extend these findings, we have sequenced a b5 immunoglobulin light chain isolated from the serum of a single domestic rabbit (2) and a b95 immunoglobulin light chain constant region from a wild rabbit (3). The b95 allotype was detected in the subpopulation oryctolagus cuniculus algirus of Zembra Island in Tunisia (4). From these sequence data, we conclude that the b95 allotype is closer to the b4, b5, and b6 ($\simeq$20% amino acid substitutions) than to the b9 allotype (36% amino acid substitutions) and that the b5 C_k region is different from the C_k region sequence previously published by Chersi et al. (5) in six residues, at least three of which correspond to polymorphic variations. However, no heterogeneity was found in the same rabbit b5 C_k region. For the b95 allotype, we have obtained in low yield a peptide which corresponds best to the region between positions 116 and 125. Two residues are different, suggesting the existence of a second b95 constant region present in the same b95 rabbit.

A comparison of the sequences of b4 rabbit kappa light chain with the b5 (97-108) region determined for the first time in this work suggest that this portion, similar to the 96-107 J region in the mouse, is encoded by an allotype specific DNA segment in the rabbit. The sequence of the b5 J region is different from the b4 and the b95 J regions at three positions. We suggest that the J_k and the C_k genes have evolved together by duplications and mutations and that the rabbit J_k-C_k gene organisation is similar to the murine λ system in which each $C\lambda$ region has its own $J\lambda$ region located on the same chromosome.

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A-2

STUDY OF THE κ1 *b* LOCUS DIVERSITY IN WILD RABBIT POPULATIONS

Amel BENAMMAR and P.-A. CAZENAVE
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In the *Oryctolagus cuniculus* species the κ light chain polymorphism appears to be much more extensive than in humans or in mice. Several allotypic specificities belonging to the κ1b series have been described in domestic rabbit (b4, b5, b6, b9, $b4^{var}$) and in wild rabbit populations of Spain (b92, b97), Portugal (b98, b99) and Tunisia (b93, b94, b95, b96). When compared to other proteins it is noted that the rabbit κ light chains share with class I MHC products the property of extreme genetic polymorphism.

On the other hand, certain experimental data suggest that at least a part of the b allotypes may be products of pseudo-allelic rather than allelic genes.

Our studies attempt to understand the evolution of the b allotypes system and the origin of their diversity. Data on the geographic distribution, the allelic frequencies, the cross-reactivities and the amino-acid sequences of these b allotypes will be discussed and some preliminary conclusions concerning the role of allotypic diversity in the rabbit, will be proposed.

A-3

ANTIBODIES AGAINST THE RABBIT κ2 LIGHT CHAIN ISOTYPE

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In a previous study, it was shown that the Basilea rabbit mutant which has lost the ability to express the b9 allotype, compensated for this lack of κ light chain, by enhanced expression of the λ light chain and of an unknown κ like chain designated κ2. It was further shown that antisera raised in rabbits against the Basilea κ2 chain, could detect very low levels of κ2 in 33% of serum samples from wild rabbits of different origins (homozygous and heterozygous at the *b* locus).

To explain these results it was proposed that all the rabbits express the κ2 isotype and that antisera raised against the κ2 chain in rabbits are in fact directed against a Bas^+ allotype of κ2 and therefore could not recognize the Bas^- κ2 allotype(s).

To test this hypothesis, polyclonal and monoclonal anti-κ2 isotype antibodies were prepared by injecting mice either with *bas/bas* κ2 reassociated light chains, or with Fab fragments isolated from a *bas/bas* homozygous rabbit. These antisera could detect the κ2 isotype in all rabbit sera (typed Bas^+ as well as Bas^-).

A-4

The molecular biology of rabbit allotypes. K. Bernstein, N. McCartney-Francis, C. B. Alexander, R. Skurla, L. Fitzmaurice, A. Pavirani, and R. G. Mage. Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

Our group has succeeded in constructing multiple cDNA clones encoding rabbit V regions. This has allowed us to study the a allotypic system in detail including the exact nucleic acid sequence of multiple a2 V regions and the partial sequence of an a1 V region. In addition, a number of sequences encoding presumed D and J regions have been obtained. Finally there is remarkably homology of rabbit and human V regions that includes the second complementarity determining region. This CDR2 sequence homology extends to human D minigenes isolated from genomic DNA and raises the question of how V region diversity is generated.

We have prepared a 60 bp restriction fragment from an a2 V region capable of distinguishing a2 mRNA from a1 mRNA on dot blots. This should allow us to answer the question: Is group a allelism due to a structural or regulatory allelism.

We have isolated and sequenced cDNAs encoding rabbit μ chains of both the secretory and membrane forms as well as a b5 kappa chain. The coding sequence for the constant region of b5 has been compared to the published DNA sequence of a b4 kappa chain and shows 85% homology. There is a marked divergence in the degree of homology between the coding region and the 3' untranslated region, with the UT being highly conserved (96% homology) between the two forms of kappa light chain.

A-5

DNA SEQUENCE OF A GENOMIC RABBIT γ-HEAVY CHAIN GENE. Steven Currier, C. L. Martens, and K. L. Knight, Department of Microbiology & Immunology, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

A recombinant phage clone containing a Cγ gene has been isolated using a cDNA probe (p2a2), synthesized from rabbit spleen poly $(A)^+$ RNA. This clone (p44) has been subjected to DNA sequence analysis and has been shown to encode the entire rabbit constant region gamma heavy chain. The C_H1, hinge, C_H2, and C_H3 gene segments are encoded in separate exons each separated by an intron ranging in size from 80 to 250 nucleotides. Comparison of the available nucleotide sequence indicates that the hinge, C_H2, and C_H3 domain encoded by p2a2 and p44 are identical. In contrast, the C_H1 domains encoded by these two clones are different. The translation of the p44 nucleotide sequence is that reported by Pratt and Mole (Biochem. J. 151, 337, 1975); the p2a2 cDNA encodes the sequence reported by Fruchter et al. (Biochem. J. 116, 249, 1970). These two amino acid sequences differ in the 3 N-terminal amino acids and in 20 of the 26 C-terminal amino acids. Thus, two Cγ chains reflecting several amino acid interchanges in the C_H1 domain are present in rabbit IgG. The presence of two different Cγ chains indicates either that there are two Cγ loci encoding rabbit Cγ heavy chain subclasses, or that there is a single Cγ locus with polymorphism at that locus.

A-6

Molecular characterization of a rabbit germline b4 J_κ-C_κ locus: an approach to the latent allotype problem. Emorine, L., Dreher, K., Max, E. & Kindt, T.J.

An increasing number of observations challenging the simple notion of allelic structural genes for immunoglobulins allotypes has been accumulated. Substantial data for the domestic rabbit C_κ b group allotypes (b4, $b4^v$, b5, b6, and b9) suggest that a given rabbit, in addition to his expressed "nominal" allotypes genes, possesses "latent", that is normally unexpressed, C_κ genes. To begin the investigation of this problem, we cloned and sequenced a J_κ - C_κ locus from a b4 homozygous rabbit. We showed that the cloned C region translates to a b4 protein sequence and that it is the one expressed by 12F2, a rabbit-mouse hybridoma secreting b4 light chains; for this reason it was named b4Nb4 (for nominal b4 detected in b4 rabbit). Southern blotting analysis of genomic DNA from rabbits of the five allotypes shows that the rabbit κ system is more complex than the human or mouse in that it has several b4 related sequences. One of these classes of sequences, referred to as b4A, is represented in all the allotypes and displays restriction sites different from those of the b4Nb4 gene as demonstrated by Southern blotting analysis. Another class of sequences, named b4B, has in each allotype a restriction pattern very similar to the b4Nb4 locus. In b5 DNA, an additional sequence, b4B'b5, was also detected. We also demonstrate in this work that the b4A and b4B sequences are associated with their own J region loci. These observations may point to regulatory mechanisms which, as observed in the murine λ system, lead to the preferential expression of one gene over the others. In addition, comparison between the expressed germline J sequence and amino acid sequences of b4 chains reveals a peculiarity of rabbit κ V-J recombination (compared with human and mouse) that may be related to the extensive variation observed in the length and amino acid composition of the rabbit third hypervariable region.

A-7

ISOLATION AND CHARACTERIZATION OF A RABBIT GENOMIC V_H GENE SEGMENT. James L. Gallarda, Kristine S. Gleason and Katherine L. Knight, Department of Microbiology & Immunology, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

A rabbit recombinant phage library was screened with the mouse S107 probe, for clones encoding V_H gene segments. Of the several positive clones detected, one, 24-3, was purified and a restriction map was determined. This clone appears to have at least three distinct segments homologous to the mouse V_H probe. Three Eco R1 fragments, each containing a sequence homologous to the mouse V_H probe were subcloned into a plasmid vector (pUC8). One of these subclones, pRV14, has been subjected to DNA sequence analysis and has been found to contain a 500 base pair sequence which encodes a V_H gene segment, including the leader sequence. This V_H gene segment has several unusual features: 1) The transcription regulatory consensus sequence $\overset{C}{T}ATA\overset{T}{A}AG$ is not found within 100 base pairs of the cap site; 2) A TGA stop codon is found in frame immediately after the codon encoding amino acid 100; 3) The V-D splice sequences in the 3' flanking region have been juxtaposed, thereby deleting the 21 base pair spacer segment which, in both mouse and human V_H genes, is normally retained. These data suggest that the V_H gene segment in pRV14 is a pseudogene. We are presently sequencing the other two subclones to determine the nature of these V_H genes.

A-8

RABBIT IgM. I. LOCALIZATION OF n LOCUS ALLOTYPIC SPECIFICITIES.
II. INDUCTION AND CHARACTERIZATION OF MONOCLONAL Ab.
Alice Gilman-Sachs and Sheldon Dray. Univ. Illinois at Chicago, Health Sci. Ctr., Chicago, IL 60612.

The localization of Cμn locus allotypic specificities to the Fabμ (CH1, CH2) or Fcμ (CH3, CH4) regions of rabbit IgM was determined by allotype analysis of trypsin digestion products. When IgM was digested with 10 percent trypsin at 56°C for 24 hr and then gel filtered through Sephacryl S300, three peaks were resolved. The peaks were characterized by molecular weight estimation and analysis with anti-a VH, anti-b and anti-Fcμ Ab; peak 1 was Fc5μ, peak 2 was Fabμ, and peak 3 contained peptides. However, when IgM was digested at 37°C, peak 1 was partially digested or undigested IgM and peak 2 was Fabμ. Radiolabeled Fc5μ and Fabμ fragments were analyzed by precipitation with Ab specific for the n80, n81, n82, n83, or n87 allotypic specificities. For 5 IgM preparations, essentially no Fc5μ was bound to anti-n Ab (3-10 percent). However, variable percentages of Fabμ were bound (36-71 percent). This variability may be due to differential degradation of the CH2 domain upon digestion with trypsin. The n82 and n83 determinants are conformationally dependent on the expression of a locus determinants whereas the so-called "true" allotypic specificities, n80, n81, and n87 are not. Both the conformationally dependent and the "true" allotypic specificities localize to the Fabμ fragments and do not appear to be present on Fc5μ fragments.

In addition, each of three monoclonal Ab was characterized. One Ab, 3C1, reacted with 80-90 percent IgM molecules but not with IgG or IgA. The Ab reacted with Fabμ but not with Fcμ fragments of rabbit IgM and thus presumably reacts with a determinant in the CH1 or CH2 domain of rabbit IgM. The second monoclonal Ab, 2D6, reacted with b5 IgM, IgA or Fabμ (80-90 percent) but did not react with b4 IgM, IgA or Fabμ. Thus, this Ab apparently recognizes the b5 allotypic specificity of rabbit Ig. The third monoclonal Ab bound 25-30 percent of IgG, IgM and IgA, regardless of the allotypic specificity and thus appeared to recognize an antigenic determinant present on a subpopulation of all classes of rabbit Ig.

A-9

CAN PENTAMERS OF IgM BE COMPOSED OF NON-IDENTICAL SUBUNITS? Wayne J. Horng, Alice Gilman-Sachs and Sheldon Dray. University of Texas Medical School, Houston, TEXAS 77030 and University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

Previously, we isolated four distinct Ab populations from an anti-a1 antiserum by sequential immunoabsorption chromatography. One Ab population designated anti-a1-A Ab reacted with most, if not all, a1 Fabγ molecules and recognized an a1 determinant which appears to be common to all a1 Fabγ molecules. Each of the other Ab populations, designated anti-a1-B Ab, anti-a1-C Ab and anti-a1-D Ab, reacted with only a fraction of the a1 Fabγ molecules but the sum of the percentages of a1 Fabγ molecules approximated the percentages precipitated by anti-a1-A Ab. In the experiments reported here, the percentages of Fabγ identified by these Ab populations are as follows: a1-A, 70%; a1-B, 15%; a1-C, 10%; a1-D, 37%. Presumably, each subpopulation of a1 Ig Fabγ is encoded by a different gene segment. We have also reacted these Ab populations with undigested IgM and Fabμ. The proportions of Fabμ bearing each of these subspecificities were very similar to the percentages found for Fabγ. However, the proportions of undigested 19S IgM bearing each of these subspecificities was quite different: a1-A, 80%; a1-B, 70%; a1-C, 55%; a1-D, 75%. These results suggest the possibility that "mixed" molecules of 19S IgM exist which bear Fabμ subunits encoded by different VH gene segments. The question arises as to whether more than one VH segment is active during IgM synthesis within the same cell or whether extracellular assembly or subunit exchange of pentameric IgM can occur.

A-10

PUTATIVE ISOLATION OF RABBIT VH GENES. Scott M. Laster, William F. Marzluff, and Kenneth H. Roux. Departments of Biological Science and Chemistry, Florida State University, Tallahassee, FL 32306.

The major VHa subgroup normally accounts for 75-99% of rabbit VH gene expression. However, rabbits prevented from expressing these gene products remain healthy and thrive under laboratory conditions. The compensating VHa-negative (minor) subgroups function competently and appear to supply a sufficiently diverse VH repertoire. In addition, we have reported that in normal rabbits the minor VHy subgroup is capable of response to a variety of antigens at levels generally proportional to preimmune expression. We have, however, detected differences between the VHa and VHy subgroups in the production of non-Ab Ig following immunization. These and other observations (e.g. latent allotypy) suggest that the normal phenotypic ratios of VH subgroup expression may not be direct result of the number of genes within each subgroup, but may be due to regulatory phenomena at the genetic and cellular level. Questions concerning quantitation and organization of the VH region genes can best be resolved by direct genomic analysis.

Using a cloned mouse VH gene (S107) as a probe, we have isolated five strongly hybridizing recombinant phage from a rabbit genomic library. These isolates have been plaque-purified to homogeneity. Each insert displays a unique EcoRI restriction pattern. Following subcloning, differential hybridization to lymphocyte mRNA from normal and suppressed rabbits of defined VH allotypes will be used to assign allotype and subgroup specificity to each isolate. Based on these results, the subcloned fragments will be used to probe genomic DNA from rabbits of known haplotype. These fragments will also be subjected to sequence analysis.

A-11

Use of allotype-defined probes for comparative studies of mRNAs from spleens and cultured cells of rabbits with defined a and b allotypes. N. McCartney-Francis, C. Alexander, K. Bernstein, L. Fitzmaurice, F. Jacobsen, A. Pavirani, R. Skurla, G. O. Young-Cooper; and R. G. Mage. Laboratory of Immunology, NIAID, NIH, Bethesda, Maryland 20205

Molecular genetic techniques are being used to study the organization and regulated expression of rabbit Ig genes. The unexpected expression of "latent" allotypes has raised questions concerning the allelism of the structural genes for rabbit a (V_H) and b (κ light chain) allotypes. Recent DNA analyses (Rougeon et al., Emorine et al.) suggest that rabbits possess multiple C_κ genes. Our laboratory has now identified and sequenced b5 κ L chain cDNAs ($p\kappa_{b5}$-1125 and $p\kappa_{b5}$-F2), a μ heavy chain cDNA encoding an a2 V_H region ($p\mu 3$), and a γ heavy chain cDNA encoding part of an a1 V_H region ($p\gamma 5$-a1) (see abstract by Bernstein et al.). Allotype-specific fragments have been generated from restriction enzyme digests of these cloned cDNAs to probe RNA dot blots. Using low stringency conditions of hybridization and washing, the probes will cross-hybridize with mRNAs encoding the various allotypes and yield semiquantitative estimates of the relative amounts of mRNA present in each preparation. Under high stringency conditions we are able to distinguish b5 and b9 poly(A)$^+$ mRNAs and cytoplasmic RNAs from rabbit splenic lymphocytes. In similar fashion, a V_H fragment probe distinguishes a2 from a1 and a3 RNA preparations. Thus, these cDNA probes allow us to identify allotype-specific mRNAs expressed by stimulated rabbit cells from short and long-term cultures. Long-term cultures consist of LPS-stimulated rabbit splenic lymphocytes which have been cloned in soft agar and maintained for over a year in culture with growth factor and LPS. Lines derived from b5 and b9 animals have been subcloned and are being characterized at the protein and nucleic acid level.

A-12

Genetics and expression of kappa and lambda light chains in BASILEA rabbits. N. McCartney-Francis, G. O. Young-Cooper, L. Fitzmaurice, A. Pavirani and R. G. Mage. Laboratory of Immunology, NIAID, NIH, Bethesda, MD 20205

A colony of Basilea rabbits was established from 6 males given to us by Dr. A. Kelus, Basel Institute for Immunology, Basel, Switzerland. During colony development, antisera that detect the bas kappa light chain were used to type the offspring of F2 and backcross matings. The segregation data are consonant with allelism (or pseudoallelism) of the gene for bas allotype with b4, b5, and b6. Splenic poly(A)$^+$ mRNA has been prepared from bas rabbits and translated in an in vitro system. Precipitations with anti-bas and anti-lambda reagents reveal two light chain species with different electrophoretic mobilities. Northern blot analyses of the bas mRNA also reveal two distinct light chain RNA species: kappa with the expected size of 1.15 kb and lambda of 1.04 kb size. Poly(A)$^+$ RNAs of the various b allotypes have been compared on RNA dot blots, using a variety of kappa cDNA probes. Under conditions for cross-hybridization, the poly(A)$^+$ RNA from bas rabbits was found to contain amounts of kappa-specific message comparable to the amounts in preparations from rabbits of the other b allotypes. At high stringency, sequence differences between the mRNAs were revealed and sequence similarities between b9 and bas were suggested.

A-13

Title : SUPPRESSION OF AN ALLOTYPIC VARIANT OF THE a_1 SPECIFICITY. ALLOTYPIC VARIANTS OF THE "a" SPECIFICITIES ARE ENCODED BY GERM LINE GENES.

Authors : B. Mariamé, O. Leo, J-D Franssen and J. Urbain.

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Allotypic markers of the "a" series have been widely used since their discovery by J.Oudin,as useful tools to study the genetics of V_H genes in the rabbit. Their interest has now increased thanks the phenomenon of allotypic variants: i.e. all molecules of the same "a" specificity are not allotypically identical, although similar and strongly cross reactive.
In order to investigate if allotypic variants are products of different germ line genes and are not arising by a soma tic diversification phenomenon, we have submitted heterozygous a1/a2 neonates (born from mating between homozygous a2/a2 female with a1/a1 male) to allotypic suppression of a avriant of the a1 specificity.
Results show this suppression works perfectly, either by maternal influence only or by neonatal treatment with an immune serum a2/a2 anti-a3 (shown by Brezin and Cazenave to recognize a subpopulation of a1 molecules). This suppression is rather short lived and does not affect the whole a1 specificity whose level is not modified.
This suggests that allotypic variants which are always expressed together are encoded by distinct germ line genes.

A-14

Title : DESCRIPTION OF A RABBIT BEARING AN ABNORMAL a3 SPECIFICITY.

Authors : B. Mariamé and J. Urbain.

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A female rabbit, originally typed heterozygous a1/a3 has been shown to bear an abnormal a3 specificity. This is illustrated by the following findings :

- the normal "peckingorder" (a1 a3) is not respected at the IgG level and the ratio between a1 and a3 IgG molecules is more or less equal to 1.
- The a3 specificity has not been detected on IgM or only in very limited amounts.
- a3 IgG had a normal L chains IEF pattern, while heavy chains were completely homogeneous (1 band).
- Moreover, this "heavy chain " has been shown by two dimension electrophoresis to be of lower molecular weight than normal.
- Finally, sera raised against these particular a3 molecules seem to recognize only 80 % of normal a3 molecules.

These findings will be discussed as regard to recombination events which are necessary to create a complete immunoglobulin gene.

A-15

C.S.Rao and S. Dubiski, Institute of Immunology, University of Toronto. Monoclonal antibodies directed against 'hidden' determinants of light chains.
In an attempt to raise monoclonal antibodies directed against allotypic determinants mice were immunized with isolated rabbit immunoglobulin light chains. The hybridoma clones were screened by the reactions of the culture supernatants with sheep red cells conjugated with immunoglobulin light chains. Three hybridoma lines were isolated, all of them reacting only with isolated light chains, but not with intact immunoglobulins. These monoclonal antibodies presumably react with some 'individual' or 'allotypic' determinants, since their reactions show some selectiveness.

Characteristics of monoclonal antibodies raised by immunization with isolated light chains

Imunizing antigen	Reacts with isolated light chains of allotypes b4	b5	b6	b9	bas	lambda*)	Molecular characteristic
b4	+	+	+	+	-	-	7S
b9	+	-	-	+	-	-	7S
lambda*)	-	-	-	-	-	+	19S

*)From suppressed Basilea homozygotes

A-16

ELECTRON MICROSCOPY OF THE RABBIT VHa ALLOTYPES. Kenneth H. Roux. Department of Biological Science, Florida State University, Tallahassee, FL 32306.

Several lines of evidence suggest that the VHa allotypes are each composed of a number of epitopes. Amino acid sequencing reveals several amino acid substitutions which, when applied to a three dimensional model, are scattered over the surface of the VH domain. In order to quantitate and determine the approximate topographical location of these epitopes, immune complexes consisting of IgG and Fab anti-VHa allotype Ab were visualized by electron microscopy. In a previous study using a 3:1 ratio of Ab to a1 IgG, one Fab Ab was observed to bind to each Fab arm of the IgG. In this study, IgG was incubated with a 20-fold excess of anti-allotype Fab Ab to assure maximum saturation of the VHa epitopes. The resulting immune complexes were separated from unreacted IgG and Fab by Sephadex G-150 column chromatography. Immune complexes were diluted to 1-10 ug/ml and prepared for electron microscopy as previously described. Analysis of the a1 allotype revealed that each arm of the IgG molecule complexed with two Fab antibody molecules. In all cases, the two Fab antibodies bound to opposite sides of the Fab arm at approximately right angles to the Fab arm of the IgG molecule. Thus, there are at least two a1 epitopes, one on either side of the VH region. Taken together, these and our previous results suggest that the anti-a1 preparation used contained one major and one minor population since only at a 20-fold excess of Ab was binding to the second epitope observed. Preliminary analysis of a2 IgG reacted with excess Fab anti-a2 Ab provided similar results.

A-17

PREPARATION AND CHARACTERIZATION OF RABBIT-MOUSE HYBRIDOMAS. John A. Sogn, Mei-chang Kuo* and Thomas J. Kindt. NIAID, NIH, Bethesda, MD 20205

Rabbit-mouse hybridomas secreting rabbit immunoglobulin chains have been used as sources of monoclonal Ig chains and mRNA in the study of rabbit Ig gene expression. With assay techniques of appropriate selectivity, the hybridoma technique can also be used to prepare relatively stable hybridomas secreting functional rabbit antibody and to prepare rabbit Ig or Ig chains with desired unusual properties. In order to prepare antibody secreting hybridomas, a rabbit of allotype a^1a^2/b^5b^5 was immunized with a short course of group C streptococcal vaccine and splenocytes from the rabbit were fused with P3X63-Ag8-U1. Screening of resulting hybrid colonies revealed seven clones secreting rabbit anti-strep antibody. Cloning and expansion of one of these seven wells showed that the secreted antibody expressed allotypes a1 and b5. Absorption with anti-a1 or anti-b5 removed antibody activity and absorption with streptococcal vaccine removed a1 and b5 reactivity. A hybridoma previously prepared from a rabbit of allotype a^2a^2/b^4b^5 and selected for secretion of an a-negative rabbit H chain has now been partially characterized. The H chain is of normal size and possesses appropriate CNBr cleavage sites. In chain recombination studies, the H chain forms 7S recombinants with b4 and b5 rabbit L chains as well as with the mouse L chain with which it is normally paired. Regardless of recombination, the H chain remains unreactive in ELISA or RIA with antisera to a1, a2 and a3. However, when the amino terminus is deblocked with pyroglutamyl aminopeptidase, the radiosequence obtained is compatible only with the consensus a2 sequence and not with a consensus a-negative sequence. Further studies on the structure of this chain should improve our understanding of the structural correlates of group a allotypy. Karyotypic studies have been carried out with a number of hybrid lines without identifying any intact rabbit chromosomes. Studies are currently in progress with a set of hybridomas secreting b4 L chains to determine the chromosomal location of the rabbit L chain genes using a probe detecting the b4 constant region and the technique of in situ hybridization.

*Fellow of the Arthritis Foundation

B. CONTROL OF ALLOTYPE AND IDIOTYPE EXPRESSION

B-1

Allotype Suppression by Autoantiallotype Antibodies in the Rabbit
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Suppression of synthesis of immunoglobulin allotypes of the a & b loci can be induced by maternal transmission of anti-allotype antibodies and/or injection of similar antibodies neonatally. Chronically suppressed rabbits can be induced to synthesise high titres of auto-antiallotype antibodies and the following are the major findings in the system: autoantibodies are clonally restricted; once induced their synthesis becomes independent of external sources of target allotype and rabbits in synthesis can maintain suppression for years or indefinitely. Rabbits that 'break' suppression after auto-antiallotype production do so with clonally-restricted molecules that lack binding activity to the host's auto-antibody and usually to that of siblings made under the same routine, and yet these are genuine allotype-positive molecules as determined by conventional typing sera. Analysis of 'escaping' allotype molecules reveals levels of complexity of allotype determinant expression that supports the view that more than one gene may code for the alleles of (at least) the b locus. Analysis of the specificity of auto-antiallotype antibodies shows that these have a restricted epitope range as well as clonal simplicity.

Recently we have persued the expression of restricted allotype determinant sets on molecules by using auto-antiallotype antibodies for suppression within 'families' of rabbits - ie by using maternal or sibling auto-antibody to suppress, and the paternal source of target allotype to produce the auto-antibody response in the suppressed offspring that is used to sustain (and dictate the specificity of) the suppression. Results of this work confirm that allotypic determinants of one allele (of the b locus in this case) may not all be represented on the same Ig molecules. Some rabbits have been manipulated this way so that they regularly co-express auto-antiallotype antibody to some (restricted set) determinants of one (b6) allele whilst synthesising other molecules that are positive for the same allele but lack the target epitopes of the auto-antibody altogether. The question as to whether the rabbit can synthesise b6 (K chain) auto-anti-b6 (K chain) is under investigation.

B-2

S. Dubiski, L. Charpentier and C. S. Rao, Institute of Immunology, University of Toronto. Antibodies to kappa isotypes raised in Basilea-suppressed homozygotes.

Rabbits homozygous with respect to Basilea gene were injected with large amounts of rabbit anti-Basilea antibody during the neonatal period. The treatment resulted in suppression of the synthesis of Basilea-positive immunoglobulins. Completely suppressed rabbits were given a series of immunizing injections consisting of Basilea-positive immunoglobulins complexed with Proteus organisms. A large proportion of animals responded by the production of precipitating antibodies reactive with Basilea-positive immunoglobulins as well as with immunoglobulins of all conventional Ab allotypes. The antisera do not react with sera of suppressed Basilea homozygotes, irrespective of their heavy chain allotypes. The antisera react with two IgG species, often forming two distinct precipitation lines with the normal sera used as antigens. The proportion of these two components in normal sera varies and is correlated with the Ab allotype. Presented evidence will show that the antisera contain antibodies reacting with two kappa subtypes, that both of these subtypes are present in normal rabbit sera and that the proportions of these isotypes depends on the kappa allotypic genotype.

B-3

BI-DIRECTIONAL PRODUCTION OF BOTH ANTI-IDIOTYPE AB AND A "LATENT" a2 ALLOTYPE. Dori S. Kazdin, Sheldon Dray, and Wayne J. Horng. Univ. of Illinois Health Science Center, Chicago, Illinois. 60612.

Previously, we induced anti-idiotype Ab (anti-IdC Ab) to a common idiotypic specificity of anti-a2 Ab prepared in $\underline{a}^1\underline{a}^1\underline{b}5\underline{b}5$ rabbits. Most of the anti-IdC Ab molecules from several a1 rabbits reacted with anti-a2 Ab. However, only 0-57% of these molecules reacted with anti-a1, anti-x32, and anti-y33 Ab. Thus, immunization of a1 rabbits with anti-a2 Ab induced a population of molecules lacking a known VH allotypic marker. We explored the possibility that these molecules bear a "latent" a2 marker. Anti-IdC Ab from an $\underline{a}^1\underline{a}^1\underline{b}5\underline{b}5$ rabbit was isolated by binding to, and elution from, an IdC Ab immunoadsorbent. The isolated anti-IdC Ab was further fractionated into a1 and non-a1 populations by using an anti-a1 Ab immunoadsorbent. When the non-a1 anti-IdC Ab was used to immunize $\underline{a}^1\underline{a}^1\underline{b}5\underline{b}5$ and $\underline{a}^3\underline{a}^3\underline{b}5\underline{b}5$ rabbits, anti-a2 Ab was produced. When an $\underline{a}^2\underline{a}^2\underline{b}5\underline{b}5$ rabbit was immunized with the non-a1 anti-IdC Ab, an anti-idiotypic antibody was produced. These data suggest that the non-a1 anti-IdC Ab bears a "latent" a2 marker. Recombinant molecules comprised of heavy chains from the anti-IdC Ab and b4 light chains of normal IgG were prepared. Anti-a2 Ab reacted with 43% of the recombinants prepared from a1 anti-IdC Ab and with 69% of those prepared from non-a1 anti-IdC Ab. These data suggest that at least 26% of the non-a1 recombinants have a "latent" a2 marker. In additional experiments, both the a1 and non-a1 fractions of anti-IdC Ab were used to inhibit the binding of ^{125}I-a2 IgG to anti-a2 antisera. When the non-a1 anti-IdC Ab was used to inhibit this reaction, the curve obtained was superimposable with those obtained using normal a2 IgG as the inhibitor. This suggests that the determinants detected on non-a1 anti-IdC Ab by anti-a2 Ab are similar, if not identical to those detected on a2 IgG by anti-a2 Ab. In contrast, while the a1 anti-IdC Ab inhibited the binding of ^{125}I-a2 IgG to anti-a2 antisera completely, the curve obtained was not superimposable with those obtained using non-a1 anti-IdC Ab or a2 IgG as the inhibitor. Thus, these data indicate that the idiotype provided a <u>bi-directional</u> stimulus to produce both anti-IdC Ab and a "latent" a2 allotype.

B-4

PRODUCTION AND IDIOTYPIC ANALYSIS OF ANTIBODY IN HOMOZYGOUS VHa-SUPPRESSED RABBITS. Scott M. Laster and Kenneth H. Roux. Department of Biological Science, Florida State University, Tallahassee, FL 32306.

We are currently examining the production and idiotypic nature of Ab found in homozygous rabbits suppressed for the expression of the VHa subgroup allotypes. Idiotypic cross-reactions between Ag-specific Ab of a given VH subgroup may indicate the presence of shared, subgroup-specific hypervariable regions. In addition, the degree of idiotypic cross-reactivity may provide some measure of the number of Ag-specific VH genes within the minor subgroups.

Neonatally suppressed VHa2 rabbits (E or F-I haplotype) were immunized with bovine serum albumin (BSA) and subsequently boosted following escape from allotype suppression. After an additional six month rest period, rabbits were again boosted with the same antigen. These rabbits produced levels of serum Ab comparable to control (non-suppressed) rabbits. The suppressed rabbits displayed patterns of clonal dominance since both primary and secondary anti-BSA populations were composed of entirely VHa-negative Ig. Even after the rest period, the Abs were exclusively VHa-negative, although levels of a2 Ig in the non-Ab population had rebounded considerably. Ability to react with albumins from other species was also compared, and in each case anti-BSA Ab from both normal and suppressed rabbits showed similar patterns of cross-reactivity. These results support our previous observations on the complex, polyclonal nature of the VHa-negative Ab response.

Affinity purified Ab from these rabbits have been used to produce six anti-idiotypic antisera. Our preliminary results indicate that these reagents recognize 20-40% of the immunogen and do not cross-react extensively with other normal or suppressed anti-BSA Ab. One anti-Id antiserum does, however, recognize the Ab produced by all members of one litter, suggesting the common expression of a shared genetic element. (Supported by NIH Grant AI 16596)

B-5

IDIOTYPIC CHARACTERIZATION OF A MONOCLONAL ANTI-a1 ANTIBODY. Dennis W. Metzger, Robin F. Renner, and Roseann Katruska. St. Jude Children's Research Hospital, Memphis, TN 38101.

An IgG1 monoclonal antibody, termed 3-2F1, has been obtained by fusing Sp2/0 cells with spleen cells from mice hyperimmunized with pooled rabbit Ig. This antibody appears to be specific for the rabbit V_H a1 allotype since binding of ^{125}I-a1 Ig to 3-2F1 can be inhibited by various, unlabeled a1 IgGs or Fab, regardless of light chain allotype, but not by a2 or a3 IgGs. That 3-2F1 recognizes most, if not all, a1-bearing molecules is shown by its ability to: (1) react with an amount of a1 Ig similar to the amount reactive with rabbit anti-a1; and (2) completely inhibit the binding of rabbit anti-a1 to a1 Ig.

It was previously demonstrated that rabbit anti-a1 antibodies bear a predominant idiotype (IdX-a1) (Metzger and Roux, J. Immunol. 129:1138). It now has been found, using anti-IdX-a1 to block antigen binding, that 3-2F1 also expresses this idiotype. In fact, 3-2F1 appears to display the entire set of rabbit IdX-a1 determinants since it will completely inhibit the reaction between rabbit anti-a1 and anti-IdX-a1. Taken together, these data strongly suggest that the anti-IdX-a1 reagent, rather than being a classical anti-idiotype, actually contains an a1-like epitope, thereby allowing it to react with almost all anti-a1 antibodies. (Supported by CORE Grant CA 21765, and by ALSAC)

B-6

REGULATION OF VHa ALLOTYPE EXPRESSION IN a1a2 HETEROZYGOUS RABBITS BY TRANSFER OF SPLENIC LYMPHOCYTES. Karen L. Muth, Robert G. Tissot and Sheldon Dray. Department of Microbiology and Immunology and the Center for Genetics, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

We have attempted to alter the expression of VHa locus allotypes in a1a2 heterozygous rabbits by transfer of splenic lymphocytes, either unseparated or B-cell enriched (<90%), from the a2a2 homozygous male parent into a1a2 heterozygous neonates within the first 24 hr post-partum. Serum a1 and a2 levels were monitored by radial immunodiffusion starting at 4 wk of age and continuing at 2 wk intervals thereafter. Serum from neonates which received 1 x 10^8 unseparated splenic lymphocytes contained significantly less a1 Ig as compared to the normal controls, whereas the serum a2 levels in the recipient rabbits were significantly increased. As a result, the serum a1/a2 ratio of the recipient rabbits was decreased for up to 32 wk and total Ig levels remained normal. It is possible that the effects observed were due to one of the following mechanisms. Transfer of mature a2-producing B-cells altered B-cell homeostasis in the neonate and may have caused a decrease in the development of a1-producing B-cells. Alternatively, the unseparated lymphocytes may contain a population of regulatory cells with suppressive activity for the a1 allotype. To examine this question, B-cell enriched lymphocytes from the a2a2 male parent were injected into the a1a2 neonates. The recipient rabbits showed increased serum a2 levels as compared to the control rabbit; however, no decrease in a1 was observed. These results suggest that a regulatory cell present in the unseparated spleen cell population, most likely a T-suppressor cell, mediated the reduction in a1 levels. To directly test this hypothesis, experiments in which purified T-cells from the a2a2 parent are transferred to a1a2 neonates are in progress.

B-7

NATURAL AUTO-ANTI-IDIOTYPIC REGULATION OF SPONTANEOUS ANTI-ALLOTYPE RESPONSES.
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This study was designed to determine if natural immune responses could elicit immunoregulatory auto-anti-idiotypic antibodies. Female rabbits heterozygous at the a and b Ig loci were bred to homozygous males. Offspring of one such breeding were studied for natural production of antibodies specific for the non-inherited allotypes and for the production of auto-anti-idiotypic antibodies. All offspring mounted natural anti-allotype responses. The anti-a1 responses cycled as a function of time but anti-b5 responses were invariant. Anti-a1 responses from 2 offspring changed specificity for different a1 subsets as they cycled. Anti-a1 was purified from the first cycle and was used in a radioimmuno-assay in an attempt to detect auto-anti-idiotypic antibody responses specific for anti-a1 idiotopes. Auto-anti-idiotypic antibodies were detected in these assays and were noted to cycle in an inverse way with the anti-a1 cycles. The idiotopes detected using the natural auto-anti-idiotypic antisera were highly cross-reactive. Subsequent deliverate immunization showed that antibodies specific for all a1 subsets could be elicited after auto-anti-idiotypic regulation had functioned showing once again the reversibility of auto-anti-idiotypic suppression in the adult. The data support the interpretation that idiotype network interactions function in naturally occurring immunologic situations and are not merely laboratory curiosities of artifacts. Supported, in part, by NSF grant PCM79-21110 and NIH grant CA23709.

B-8

IDIOTYPIC INTERNAL IMAGES OF RABBIT b ALLOTOPES

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The analysis of idiotypy of rabbit antibodies directed against rabbit allotypes of the b series has revealed an apparent recurrence of anti-allotypic idiotypes raised in outbred rabbits. These findings were extended in other laboratories to antibodies directed against allotypes of the a series.

To explain the recurrence of rabbit anti-b4 and anti-b6 anti-allotypic antibodies we propose that some anti-idiotypic antibodies (Ab2) directed against anti-allotypic antibodies (Ab1) carry internal images of the original immunizing allotype. The recurrence observed in these systems could result from the combination of the different Ab1 with internal images of b allotopes borne by Ab2β anti-idiotypic antibodies. This was shown by studying Ab3 antibodies raised against Ab2β . Using mouse monoclonal antibodies we have shown that internal images of b6 rabbit allotopes are, in this species, associated with internal images of rabbit κ1 isotypic determinants.

The parallel evolution of b allotopes in the rabbit and their internal images in the mouse will be discussed.

B-9

DO RABBITS AND NON-RABBIT SPECIES EXPRESS SIMILAR CROSSREACTIVE IDIOTYPES ON ANTI-ALLOTYPE ANTIBODY? Kenneth H. Roux and Dennis W. Metzger. Dept. of Biological Science, Florida State Univ., Tallahassee, FL 32306 and St. Jude Children's Research Hospital, Div. of Immunology, Memphis, TN 38101.

Several laboratories have recently reported a high degree of IdX on rabbit anti-allotype Ab. One interpretation of this data is that most or all rabbits possess similar highly conserved genes for the production of IdX anti-allotype Ab. Alternatively, sterically or serologically similar idiotypes may be the products of unrelated genes. To test these hypotheses, anti-allotype (al) Ab was induced in several other species and tested for its ability to inhibit a rabbit Id-anti-Id reaction. Guinea pigs, mice, goats, and chickens were immunized with al IgG and the resulting antisera extensively adsorbed to remove anti-isotype Ab. Anti-al Ab was purified by immunoadsorption and tested for IdX expression by solid-phase RIA. Each of the preparations was able to inhibit the reaction. Maximum inhibitions ranged from 80-100%. Comparison to a rabbit anti-al standard revealed that from 1-560 times as much anti-al Ab from other species was needed to produce 50% inhibition. One guinea pig Ab, which expressed the IdX to the same degree as rabbit anti-al Ab, also gave a distinct precipitin band against rabbit anti-Id Ab by immunodiffusion. Controls for leaching of al from the immunoadsorbents showed no inhibition in RIA reactions. Each anti-al preparation was also tested and found to be inhibitory in an al-anti-al solid-phase RIA. These results weaken the interpretation that the ubiquitous expression of IdX reflects shared conserved genes (unless other species also possess similar conserved genes for anti-al Ab). We suggest that either (1) the al epitopes induce Ab which possess high fidelity negative images of the al epitopes or (2) the anti-Id Ab used in these experiments represents high fidelity positive images of the al epitopes. In the latter case, any anti-al Ab would be inhibitory in the above competition experiments.

B-10

Title :SIMULTANEOUS EXPRESSION OF AN IDIOTYPIC COLLECTION IN A SINGLE RABBIT.

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The immunization cascade has revealed that outbred rabbits may be programmed to express any idiotype taken from a non-related animal. Therefore, all individuals of the species seem to possess the genetic potential for the synthesis of all idiotypes of the species. Because the immunization with anti-Id antibodies can induce the expression of a silent idiotype, we have attempted to induce in a single individual the simultaneous expression of several usually silent clones. Using the Ab1-Ab2-Ab3 scheme of immunization, rabbits were injected with a mixture containing six different Ab2. Each anti-Id (Ab2) of the mixture was made against six non-crossreactive idiotypes (Ab1), specific for micrococcal carbohydrates. Rabbits synthesizing Ab3 received antigen in order to produce Ab1'. The results show that purified anticarbohydrate antibodies from Ab1' sera were reactive with each member of the Ab2 mixture. Therefore, Ab1' shared idiotypes with the series of Ab1 initially expressed by different rabbits. The idiotypes expressed within Ab1' were present on different antibody molecules. No Ab1' subfraction possessed more than one of the imposed idiotypic specificity. These experiments indicate the presence of independent idiotypic regulatory circuits. By suitable idiotypic manipulations, several independent silent clones can simultaneously be relieved from suppression. Moreover, the experimental approach used offers the possibility to determine the size of the repertoire of a species for a given antibody specificity.

B-11

Latent immunoglobulin allotype in normal and Trypanosoma brucei - infected rabbits. Benjamin Wolf, Sally Tonkin, and Juanita Yee-Foon, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104

Although nominal immunoglobulin allotypes appear to be encoded by codominant allelic genes, a number of laboratories including our own, have described the existence of rabbits which exhibit additional or latent non-allelic allotypes in the serum or in B cells. In our breeding stock of rabbits, we selected sera from two animals which displayed latent a1 and e14, markers for V_H and F_c regions in IgG. The a1-bearing molecules were purified from an anti-a1 immunoadsorbent column and were found to contain d12 in the hinge region as well as e14 in the F_c region, after proteolytic enzyme digestion. This marks the first time that a latent haplotype, with three closely linked γ chain genes, has been reported, and implies the existence of V_H-C_H joining mechanisms specific for latent markers. In other rabbits typed as nominal a1,3/b4 which did not show latent allotypes in the serum, we found that, following infection with Trypanosoma brucei, 4 of 6 animals exhibited latent a2, b5 and two of these rabbits showed b6 and b9. Substantial increases in concentration of the nominal allotypes were also detected. IgG from a serum containing latent b9 was employed to isolate light chains by partial sulfitolysis, using the method of Rejnek et al (1968). RIA confirmed that b9 was present (5μg/ml). Post-infection bleeds tested for IgM (n) allotypes from one rabbit showed n82, a specificity linked only to some a2 haplotypes. These data indicate that many of the common group a and b specificities and possibly the group n (IgM) allotypes can be activated in randomly selected rabbits. However, in comparison to the nominal (allelic) genes, the extent of activation of the latent (non-allelic) genes is markedly lower.

C. LYMPHOCYTE DIFFERENTIATION AND FUNCTION

C-1

IMMUNOLOGICAL COMPETENCE OF DONOR-DERIVED B LYMPHOCYTES IN CHIMERIC RABBITS. Frank L. Adler and Louise T. Adler, St. Jude Children's Research Hospital, Memphis, TN 38101

Spleen or lymph node cells ($2x10^8$) from adult donors injected intraperitoneally into newborn histocompatible recipients establish long lasting chimerism. The deliberate mismatching of donor and recipients with respect to Ig allotypes allows enumeration of donor derived B cells (rosetting) and measurement of Ig secreted by such cells (HI or ELISA). In the majority of chimeras, the serum content of donor type Ig stabilizes at 10-50 percent of total Ig. Antibodies produced by chimeras in response to several TI and TD antigens are generally of the recipient's allotype, suggesting that donor-derived B cells lack immunocompetence. However, if the donor had been primed to one of these antigens, participation of donor-derived cells in the response to this antigen was readily demonstrable. Further, antigen administered to the recipient from birth can be shown to drive selectively the proliferation of specifically responding B cells from an immunized donor. These observations suggest that committed donor B cells colonize the recipient, that they undergo clonal expansion, and that the apparent lack of immunocompetence of B cells from non-immune donors reflects the colonization of the recipient by a few clones of donor origin and therefore results in a greatly curtailed repertoire of donor B cells. In recipients that are mismatched so as to allow for allogeneic reactions there appears to occur a general stimulation of the lymphoid system which seems to facilitate engraftment and expansion of an enlarged repertoire of donor B cells and also of donor T cells. In such recipients the participation of donor-derived B cells in antibody responses is readily demonstrable. It is believed that chimerism induced by peripheral B lymphocytes may be a useful model for the study of the biology of committed B cells and also may provide leads for the restoration of immunocompetence in human lymphoid graft recipients. (Supported by Grants CA 23709, AI 13159, CORE Grant 21765, and by ALSAC)

C-2

ABROGATION OF ALLOTYPE SUPPRESSION IN LYMPHOID CELLS TRANSPLANTED TO HISTOINCOMPATIBLE HOSTS. Louise T. Adler, St. Jude Children's Research Hospital, Memphis, TN 38101.

Stable chimerism (75/86) followed the transfer of histocompatible (RLA-matched) spleen, lymph node, bone marrow, or thymus cells of adult rabbits into newborn recipients. The presence of functional B cells of the donor was monitored by deliberately mismatching for a and/or b locus allotypes. In contrast, death with symptoms of GvHR occurred in 18 of 22 RLA-heterozygous newborns injected with homozygous cells of a parental RLA type. However, 2 recipients of parental type cells survived and were converted to the lymphoid cell phenotype of the donor. It has been shown previously that cells from allotype-suppressed donors become engrafted in histocompatible newborn recipients but that no Ig of the suppressed type is ever produced in these animals. However, transfer of cells from a suppressed donor of the parental RLA type into RLA heterozygous newborns leads to a dramatic abrogation of allotype suppression in the engrafted cells. This is seen in survivors which undergo hematopoietic reconstitution by donor cells as well as in littermates which succumb to fatal GvHR at a few weeks of age. These results suggest that intensive antigenic stimulation as provided by the allogeneic effect in vivo interferes with the maintenance of allotype suppression. Possible mechanisms include elimination of suppressor cells and/or the activation of cellular precursors previously uncommitted to production of the suppressed allotype. [Supported by NIH Grant AI 13159, NCI Grant CA 21765 (CORE), and by ALSAC]

C-3

INDUCTION OF POLYCLONAL IMMUNOGLOBULIN PRODUCTION BY RABBIT MILK CELL SUPERNATANT. D.A. Axelrod, R.H. Reid, J.A. Wright, W.T. McCarthy, D.R. Edwards, R.Y. Dorsey, J. Tseng. Laboratory of Immunology, Dept of Gastroenterology, Division of Medicine, and Dept. of Experimental Pathology, Division of Pathology, Walter Reed Army Institute of Research, Washington, D.C. and Dept. of Pediatrics, Harvard Medical School, Boston, MA.

A substance derived from human breast milk cells has been reported to induce IgA elaboration in peripheral blood lymphoid cells. The purpose of this report is to determine the presence of a similar material in the rabbit and to study the activity of this material upon the rabbit Gut Associated Lymphoid Tissue. Milk cells were recovered from lactating rabbits and placed in 1 ml cultures for 72 hours when the supernatants were pooled. Mononuclear cells from the blood, spleen, mesenteric lymph node, Peyer's patch and lamina propria at concentrations of 2×10^6 per ml and 3×10^5/ml were exposed either to media alone or to media plus breast milk supernatant. At 72 hours supernatants were assayed for total IgA, IgG, IgM using an enzyme linked immunosorbent assay with a lower level of sensitivity of 10 ng/ml. From a mean baseline Ig concentration of 20 ng/ml increases in IgA to a mean of 120 ng/ml and increases in IgG and IgM to a mean of 220 ng/ml in peripheral blood, spleen, and Peyer's patch, but not in lamina propria or mesenteric lymph node cells were seen. Our data suggest the existence of a polyclonal B-cell activator from rabbit milk cell supernatant which has activity upon Gut Associated Lymphoid Tissue and may represent an important mechanism in the induction of the neonatal immune system.

C-4

BLAST TRANSFORMATION OF B CELLS INDUCED BY AN α-MACROGLOBULIN ASSOCIATED LYMPHOKINE PRODUCED IN CROWDED LYMPHOID CELL CULTURES. Doina Ganea, Jin-Lai Chang, Sheldon Dray and Marius Teodorescu. Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, IL. 60612.

A polyclonal B cell activator (PBA) as a helper factor has been postulated by Coutinho and Moller. Here we investigated whether during close B-T cell contact, i.e. autologous MLR, such a PBA is produced. Rabbit lymph node cells were cultured at a concentration of 10^6 cells/ml in a serum-free medium under crowded conditions to promote close cell contacts and under spread conditions to minimize cell contacts. The culture medium was replaced daily for three days and all supernatant fluids were extensively dialysed. Whereas all crowded cell supernatants (CCSup) induced ^{14}C-uridine incorporation in rabbit lymph node cell cultures, the spread cell supernatants (SCSup) were inactive. CCSup induced ^{14}C-uridine incorporation in rabbit B but not T cells. B cell blast transformation by the CCSup was not species specific since CCSup also induced blast transformation of nude mouse spleen cells. The CCSup was fractionated on a BioGel P 300 column and the ability to induce blast transformation was recovered exclusively in fraction I, containing the macroglobulins. The material was then absorbed with insolubilized goat anti-rabbit IgM (anti-μ) or anti-α macroglobulins (anti-αM). The blastogenic activity was present in the fraction absorbed with anti-μ, but not in the fraction absorbed with anti-αM, indicating that the blastogenic activity was associated with the αM. The ability to induce ^{14}C-uridine incorporation by CCSup, fraction I or fraction I absorbed with anti-IgM antibody was blocked by aprotinin, a low molecular weight protease inhibitor. However, aprotinin had no effect on the blast transformation induced by LPS or dextran sulfate. Preliminary data suggest that a low MW inhibitor of the PBA is also produced in the crowded cell cultures and is removed by extensive dialysis of the CCSup. We concluded that the αM present in rabbit lymphoid cell cultures maintained in close contact is responsible for the polyclonal stimulation of B cells and that this stimulation is due to an αM-associated lymphokine which is presumably a proteinase.

C-5

POLYCLONAL B CELL ACTIVATOR AND ITS LOW MOLECULAR WEIGHT INHIBITOR INDUCED BY INOCULATION OF ALLOGENEIC CELLS IN RABBITS. Doina Ganea, Ana Teodorescu, Sheldon Dray & Marius Teodorescu. Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, IL. 60612.

Previous results from our laboratory indicated that upon close contact in vitro, B cells stimulate T cells to produce an α-macroglobulin (αM) that behaves as a polyclonal B cell activator (PBA). To determine whether such a PBA can be induced in vivo, rabbits have been inoculated with allogeneic lymph node cells (Al-LNC) or with sheep red blood cells (SRBC). The PBA appeared and reached a maximum on day 4 after inoculation, decreased gradually after 1 week, and became undetectable at 6 to 7 weeks. The PBA titer for rabbits inoculated with Al-LNC was 100 to 1000 times higher than for rabbits inoculated with SRBC. For the rabbits inoculated with Al-LNC the IgG concentration increased in the serum to a four-fold maximum at 3-5 weeks. The PBA activity was associated entirely with the αM fraction. The αM separated from normal rabbit serum had no PBA activity, but when normal αM was complexed with trypsin, a high PBA titer was obtained. The PBA activity of the normal αM-trypsin complex and of the αM from rabbits inoculated with Al-LNC were inhibited by low MW serine protease inhibitors such as aprotinin or phenylmethylsulfonyl fluoride, but not by high MW inhibitors such as soybean trypsin inhibitor. The αM from Al-LNC injected rabbits had an enzymatic activity for p-toluene sulfonyl L-arginine methyl ester. Ultrafiltration of the serum of rabbits inoculated with Al-LNC on membranes with a cut-off of 10,000 D resulted in an increase in the PBA titer, suggesting that a low MW PBA inhibitor (LOMPIN) has been removed. The ultrafiltrate (UF) containing LOMPIN was concentrated on a membrane with a cut-off of 1,000 D. This UF inhibited the PBA activity of the serum from Al-LNC injected rabbits, as well as of the purified αM. The inhibition was dose-dependent. UF from normal rabbit serum had no effect on the PBA activity. The enzymatic activity of trypsin, as tested on radioactive fibrinogen plates, was inhibited by LOMPIN. This inhibition was dose-dependent and the inhibition curve was similar in shape with that obtained with aprotinin. We concluded that iv inoculation of allogeneic lymphoid cells in rabbits induces the appearance in the serum of a PBA, that was identified as an αM-serine protease complex, as well as of a low MW inhibitor of the PBA

C-6

EXPRESSION OF THE b9 ALLOTYPE BY PRE-B CELLS AND B LYMPHOCYTES FROM BASILEA RABBITS. W.E. Gathings, R.G. Mage and G.O. Young-Cooper. The Cellular Immunobiology Unit, University of Alabama in Birmingham, Birmingham, AL and the Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, MD.

The Basilea strain was derived from a single male rabbit which failed to express the b9 κ allotype expected from the paternal genotype. Rabbits homozygous for this trait produce little or no κ light chains but compensate by producing light chains which are almost exclusively of the λ type. We hypothesized that the Basilea mutation might represent a defect in b9 or b9-like κ chains which results in effective clonal abortion of $b9^+$ cells. In order to test this hypothesis, we employed affinity purified antibodies and immunofluorescent staining techniques to examine B lineage cells from newborn b^4b^9, b^4b^{bas} and $b^{bas}b^{bas}$ rabbits for the expression of κ light chain determinants. In the 3 groups of rabbits studied, the frequency of bone marrow pre-B cells ranged from 7.4-18.5%; approximately 10% of the total pre-B cells also stained for κ light chain allotypes. In the b^4b^9 rabbits, equal numbers of pre-B cells stained for b4 and b9. Equal frequencies of $b4^+$ and $b9^+$ pre-B cells were also observed in b^4b^{bas} heterozygotes, while only $b9^+$ pre-B cells were detectable in Basilea homozygotes. In contrast to the normal numbers of $b9^+$ pre-B cells observed in Basilea rabbits, the frequency of $b9^+$ B cells in marrow of these animals was greatly reduced, representing only 2-4% of the IgM^+ B cells in both b^4b^{bas} and $b^{bas}b^{bas}$ rabbits. Absorption of the anti-b9 conjugate with purified bas light chains had no effect on the frequency of pre-B or B cells which stained with this antibody.

These results suggest that the lesion which is responsible for the deficiency in production of immunoglobulin molecules with the b9 or b9-like determinant in Basilea rabbits is manifested by a failure in normal differentiation of $b9^+$ B lymphocytes.

C-7

COMPARISON OF Ig SECRETION AND MEMBRANE Ig EXPRESSION BY LYMPHOCYTES FROM NORMAL, ALLOTYPE-SUPPRESSED, AND CHIMERIC RABBITS. Yohachiro Ohama and Louise T. Adler, St. Jude Children's Research Hospital, Memphis, TN 38101.

Quantitative expression of secreted and membrane-bound Ig was determined in normal and allotype-suppressed rabbits heterozygous with regard to *b* group allotypic determinants. Secreted Ig was assessed in terms of serum concentrations as well as by enumeration of Ig-secreting cells (ISC) in blood and spleen using an allotype-specific reverse hemolytic plaque assay. The percentages of cells with mIg of relevant *b* group allotypes were expressed as rosette forming cells (RFC) using SRBC to which purified anti-allotypic antibody had been linked. When PBL's and spleen cells of allotype-suppressed rabbits were analyzed, the proportion of cells secreting Ig of the suppressed type was seen to be up to 50 times lower than that of cells expressing mIg of the corresponding allotype, confirming evidence previously obtained by ourselves and others for the presence in the suppressed animal of many lymphocytes which can synthesize mIg but lack secretory capacity. Lymphocytes of normal heterozygous rabbits, in contrast, do not exhibit this disproportionality between secretory and membrane-bound Ig expression, as measured by ISC, RFC, and serum Ig concentrations. Similarly, in rabbits which have been made chimeric with regard to B cells by histocompatible lymphoid cell engraftment at birth, the proportions of cells with mIg of donor and recipient types were comparable to those of cells secreting Ig of the corresponding allotypes. [Supported by Grants AI 13159 and CA 21765 (CORE) from the National Institutes of Health and by ALSAC]

C-8

The character of the binding and constant part of the T cell receptor molecule specific for antigen

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The presence of a allotypic determinants was tested in the fractions obtained by gel filtration of antigen-specific receptors isolated by immunoadsorption technique from lymphoid cells of antigen stimulated a3-3 rabbits. This technique as well as the inhibition of the reaction of isolated receptors with anti Ig⁻ receptor antisera (anti R) by anti a3 antibodies failed to demonstrate the presence of a allotypic determinants. The results suggest that T cell receptor molecule does not carry the whole VH region.

The inhibitory effect of Ig⁻ receptors isolated from the lymphoid cells of stimulated A/J mice on the cytotoxic effect of anti Ia antibodies on mouse spleen cells in the presence of rabbit complement was tested. The results showed that all preparations inhibited the cytotoxic reaction with the average effectivity of 60 %.

In order to find out whether the Ia determinants were carried by the T cell receptor molecule the anti Ia antibodies were used for inhibition of the reaction of isolated mouse receptors with anti R antisera. It has been shown that the reaction of three anti R antisera with twelve different receptor preparations were inhibited by anti Ia antibodies.

Similar results were obtained in the experiments where the reaction of rabbit specific receptors with anti R antisera directed to rabbit Ig⁻ receptors were inhibited with antisera to rabbit Ia antigens. This confirms that both rabbit and mouse T cell receptors carry MHC I region gene products.

C-9

MITOGEN STIMULATED SYNTHESIS OF IMMUNOGLOBULIN BY RABBIT LYMPHOCYTES IN VITRO. Eileen Skaletsky (University of California, San Diego) and Stewart Sell (University of Texas - Houston). Stimulation of immunoglobulin synthesis in vitro by Concanavalin A and anti-Ig in cultures of rabbit lymphoid cells has been analyzed . Qualitation of Ig levels was done by measuring the incorporation of ^{3}H-leucine into newly synthesized proteins, followed by the specific absorption of tritiated immunoglobulin by staphylococcal protein A. Concanavalin A stimulated Ig production by spleen cells only when T cells were present, but anti-immunoglobulin serum enhanced Ig synthesis in the absence of T cells. In contrast, neither Con A nor anti-immunoglobulin serum stimulated peripheral blood lymphocytes to produce enhanced levels of Ig. It was concluded that both Con A and anti-Ig do not activate resting B cells but drive differentiation of B cells which are already synthesizing Ig. Anti-Ig acts directly on B cells whereas stimulation of Ig synthesis by Con A occurs indirectly through stimulation of T cells.

C-10

INHIBITION OF THE PROLIFERATIVE RESPONSE OF RABBIT B-LYMPHOCYTES TO ANTI-LIGHT CHAIN ALLOTYPIC ANTIBODIES BY PREVENTING CELL-CELL CONTACT IN SOLID MEDIUM OF 0.5% AGAROSE. Helga Spieker-Polet & Marius Teodorescu. Univ. of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

The involvement of surface Ig of B lymphocytes in the immune response was suggested by the experiments showing that B cells can be stimulated to proliferate in liquid medium by anti-Ig-antibodies. It is also well established that a B-cell response to an antigen involves its specific binding to the surface Ig on B cells. Both the antigen and the anti-Ig antibodies have to be multivalent to be stimulatory. Therefore, the activation of B-cells may involve cross-linking of Ig-molecules on one cell, as has been proposed, or cross-linking of different cells. To determine which is the most likely mechanism, rabbit spleen and lymph node lymphocytes of the b_4 allotype were stimulated to synthesize DNA by purified anti-b_4 antibodies or by Con A. The proliferative response was greatly reduced and in many cases completely abolished if the cells were separated by immobilization in medium with 0.5% agarose at the time of stimulation. As visualized under the microscope, the cells remain largely isolated in this "solid" medium without forming clusters. If the addition of agarose is delayed by 2-5 hours the clusters can be seen and the proliferative response is only partially, if at all, inhibited. These results suggest that the function of both anti-Ig antibody and Con A is to establish cell-cell contacts by forming a bridge between different cells, rather than to cross-link receptors on the same cell. Thus, the surface Ig might not deliver any activating signal to the cell but rather the signal is provided by another Ig-bearing cell.

C-11

Disseminated infection with Malignant Rabbit Fibroma Virus: Immunohistologic Investigation of a model of immunosuppression in viral oncogenesis.

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Malignant rabbit fibroma virus (MV) was discovered in a stock of uncloned shope fibroma virus (SFV). Clinically, MV induces a unique syndrome of disseminated malignancy and secondary Gram-negative infection over the course of 10-12 days. Tissues from rabbits with MV infection were examined histologically and immunohistologically. Histologic evaluation disclosed a morphologically malignant tumor. Epithelial structures overlying this tumor showed varying degrees of atypia and squamous mataplasia. These were most pronounced in the conjunctiva and nasal mucosa, the two areas most prominently involved by Gram-negative infection. Draining lymph nodes showed increased cellularity in the diffuse cortical zone. These cells stain positively with anti-T lymphocyte reagents. Immunohistologic examination revealed that while MV only induces tumors in the superficial body parts (skin), it is detectable systemically in the fixed phagocytes of the lung, GI tract, lymph nodes, liver and elsewhere. Tumor cells contain viral antigen as does, to a small degree, overlying skin and striated muscle. Nasal epithelium and conjunctiva, in contrast, contain large amounts of MV, both in the superfical epithelium and (in the nasal mucosa) in the glands underlying the surface. Other epithelia are not affected. Rabbits bearing MV-induced tumors show a profound impairment in immunologic function as measured by T and B lymphocyte mitogen-induced blastogenesis. MV-induced viral disease may serve as a model for oncovirally-induced immunologic impairment in people.

C-12

ACTIVATION OF RABBIT T CELLS BY ANTIALLOTYPE ANTIBODIES.

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In this study we addressed the question whether, in the rabbit, T cell receptors share determinants with immunoglobulins. To this end the effect of anti-allotype antibodies on "in vitro" T cell function was analysed. Following results were obtained :

1° Resting T cells do not respond to antiallotype antibodies alone. However, upon simultaneous addition of T cell growth factors (TCGF) and antibodies, a significant proliferation is obtained, indicating that anti-allotype antibodies induce expression of TCGF receptors on rabbit T cells.

2° Anti-allotype antibodies are mitogenic for activated T cells, derived from the lymph nodes of immunized animals.

3° Anti-allotype antibodies can substitute for antigen-presenting accessory cells in the antigen-induced proliferation of primed T cells.

All these effects were observed with anti-allotype reagents(either classical isologous antisera or monoclonal hybridoma-derived antibodies) directed against the relevant a-locus (V_H region) or b-locus (kappa light chain) markers. Heterologous anti-Ig antibodies directed against the Fab portion of rabbit Ig produced similar effects, while anti-Fc or antibodies directed against irrelevant allotypic specificities were totally ineffective. Monovalent fragments were equally stimulatory for T cells as intact IgG, indicating that crosslinking of Ig-like molecules is not a necessary requirement in this activation process.
A possible interpretation of these results is that V_H as well as L-chain determinants are part of a functional receptor on rabbit T cells.

C-13

Co-suppression of nominal and latent allotypes in individual rabbit lymphocytes.
Benjamin Wolf, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA 19104

Endogenous synthesis of latent and nominal allotype was observed *in vitro* in rabbit peripheral blood lymphocytes (PBL). The PBL had been taken from rabbits displaying the nominal b4 specificity as well as the latent b5 and b6 either on the cell surface or in the serum or both. The cells were treated with pronase to remove surface allotype and cultured in serum-free medium to regenerate the nominal and latent markers. A mixed anti-globulin rosette test was developed which detected single, double, or triple expressing cells for b4, b5 and b6. For the rosette assay, the three indicator red cells were either left unstained, stained with fluorescein (green) or a combination of fluorescein plus rhodamine (orange). The red cells were then tagged with b4, b5 and b6, respectively. Cells were seen bearing the b4, b4 + b5, b4 + b6, or b4 + b5 + b6, as single, double or triple expressors. No latent allotype-bearing of nominal surface b4 with anti-b4 antibodies resulted in the co-suppression of the latent b5 and b6. In contrast, *in vitro* suppression of latent b5 and b6 with anti-b5 and anti-b6 antibodies respectively, did not cause the co-suppression of the nominal b4 allotype. Co-suppression of latent and nominal markers in the same cell confirms that the biosynthetic mechanisms are within the individual cells. Although a likely explanation for co-suppression is that the latent and nominal allotypic genes are cis-expressed on the same parental chromosome, direct evidence would need to be obtained by experiments at the gene level.

D. RABBIT LYMPHOCYTE ANTIGENS

D-1

UNEXPECTED CROSS-REACTIONS OF MONOCLONAL ANTI-RABBIT T CELL ANTIBODIES. Zhang Chen, Dennis W. Metzger, and Frank L. Adler. St. Jude Children's Research Hospital, Memphis, TN 38101.

We have produced an IgG1 pan-rabbit T cell antibody, designated ACM-1, that recognizes the same (or spatially close) epitope as the previously described IgM antibody, 9AE10 (McNicholas et al., Mol. Immunol. 18:815, 1981). These antibodies react with a protein that appears to be similar to mouse and rat Thy-1 since the antigen has a MW 25-27,000 and is present on >95% thymocytes, 50% spleen cells, 50-70% lymph node cells, and 30% bone marrow cells. Neither ACM-1 nor 9AE10 react with Ig^+ lymphocytes or peritoneal macrophages *in vitro*. In attempts to obtain *in vivo* T cell depletion, rabbits were injected i.v. with ACM-1 or 9AE10 (10 ml ascites fluid/kg). Surprisingly, there were larger decreases in total WBC number than would be expected for simple T cell depletion. *In vitro* immunofluorescence and cytotoxicity assays on enriched neutrophil populations revealed that 80-95% of this major component of blood was reactive with ACM-1 and 9AE10. Controls for neutrophil Fc binding were negative. Furthermore, quantitative absorption failed to show binding to rabbit brain. Thus, while the analogy of these monoclonal antibodies with anti-Thy-1 remains in question, a sharing of an epitope by T cells and PMN cells has been demonstrated. Binding characteristics of a series of additional anti-rabbit lymphocyte monoclonal antibodies will also be presented. (Supported by CORE Grant CA 21765, CA 23709, and by ALSAC)

D-2

Cohen, Carl; Department of Surgery, University of Illinois College of Medicine
On achieving cellular transplantability in rabbits.

Over the past years a major concern in our laboratory has been the development of the rabbit for studies in transplantation. Our first interests had been in the study of red cell antigens in a model of maternal-fetal incompatibility for the investigation of the human Rh problems. Concomitant with the blood group studies was the beginning of intensive inbreeding in the rabbit to attain the level of homozygosis sufficient to ensure compatibility for tissue transplantation. Eventually we verified the fact that the rabbit is not inherently an easily inbred species. We were confronted with the expected marked decline in fertility and fecundity, but the persistence of heterozygosis for blood group and histocompatibility genes was much greater than expected and was a major deterrent to achieving transplantability. Since we had been able to induce tolerance to red cell antigens with the injection of red cells into the newborn rabbit during our studies of tolerance in the Rh model, it seemed reasonable to try to use neonatal injections of cultured fibroblasts from the intended skin donor to induce cell antigen tolerance without the introduction of alien lymphoid cells or tissues. This procedure proved to be feasible for skin grafting and revealed some information about changes in cells in culture. With the success in the induction of the chimera by Drs. Louise and Frank Adler, we turned to this system to study the role of the histocompatibility genes on chimera formation and the GVH reaction. Our continued work on the inbred model for transplantation allowed us to compare breeding systems and skin graft survival in grafts between relatives. We devised one scheme using already transplantable rabbits to produce a fairly large number of animals in which cross grafting gave sufficient extension of graft survival to sustain studies on the transfer of cells. Prolongation of the skin graft survival in these various models can be compared and their potential for cellular immunity studies evaluated.

D-3

RABBIT LEUKOCYTE SURFACE MARKERS DEFINED BY MONOCLONAL ANTIBODIES.

W. De Smet, M. Vaeck, E. Smet, L. Brys, and R. Hamers.
Algemene Biologie, Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, Paardenstraat 65, 1640 St-Genesius-Rode, Belgium.

As the reagents for typing immunocompetent cells in the rabbit are grossly lacking, we have developed several monoclonal antibodies (McAb) against rabbit leukocytes and characterized them in binding and functional studies. McAb 1.24 stains thymocytes, bone marrow cells, peripheral T and B cells and blood monocytes. T cells express more 1.24 antigen than B cells. In the absence of added complement (C), McAb 1.24 inhibits alloantigen-, Concanavaline A (Con A)-, and phytohemagglutinin (PHA)-, but not pokeweed mitogen (PWM)- or anti-immunoglobulin-induced cell proliferation. It also strongly blocks anti-sheep erythrocyte plaque forming cell responses. A second McAb, designated 4.B9, binds to 20% of thymocytes and to most, if not all, peripheral T cells and "in vitro" activated T cell blasts. A third one, 10.B3, is reactive with the nearly entire thymocyte and a major peripheral T cell population. Two-colour membrane immunofluorescence experiments reveal the presence of a minority of weakly stained $4.B9^+$ Ig^+ and $10.B3^+$ Ig^+ cells in peripheral blood leukocyte suspensions. Without C, both McAb inhibit Con A- and PHA-induced mitogenesis, but have no effect on PWM-, antigen-, or alloantigen-induced cell proliferation. A fourth McAb, 12.C7, binds to 60% of thymocytes and to 10-30% of peripheral T lymphocytes at high level fluorescence. T cell blasts obtained in mixed leukocyte reactions are partially stained by McAb 12.C7, while those obtained after Con A or PHA activation are not. In addition, McAb 12.C7 is completely unreactive with B cells or monocytes. Without C, it does not seem to interfere with any of the "in vitro" functions tested. On the other hand, lysis treatment with 12.C7 and C eliminates most of the alloantigen reactive T cells. All antigens studied here do not appear to be expressed in nonleukon tissues as they do not bind to erythrocytes and are absent from brain, heart, liver and kidney by quantitative absorption analysis.

D-4

Separation of Rabbit T and B Cells for Determination of T-Cell Function

Susan Jackson, Debra L. Wetterskog & Thomas J. Kindt, Laboratory of Immunogenetics, NIAID, NIH, Bethesda, Md. 20205

We have recently described a method for the direct removal of T lymphocytes by "panning" of rabbit splenocytes on plastic dishes coated with L11/135, a monoclonal reagent specific for all rabbit T cells (J. Exp. Med. 157:34, 1983). This simple technique allowed the effective removal of T cells from a mixed population of rabbit splenocytes; however, due to the high density of the determinant recognized by L11/135 on T lymphocytes, it was not possible to remove the tightly bound cells from the plastic dishes, even by mechanical manipulations or enzyme treatment. We have now developed a method for removal of T cells bound to antibody-coated dishes by competition with excess L11/135 antibody in solution. B cells have been further purified in a similar manner: nonadherent cells from L11/135-coated plates were plated onto a dish coated with anti-rabbit Ig, and removed by competition with anti-rabbit Ig in solution after adherence to this plate. Using these methods, T and B lymphocytes from spleens of animals hyperimmunized with Group C streptoccal vaccine have been separated. The separated T and B cell populations were not cross-contaminated, as shown by the functional criteria of: 1) mitogen response assays, and 2) specific antibody production determined by an ELISA designed to detect antibodies directed at the group-specific carbohydrate of Group C streptococcus. There was no significant production of group-specific antibody by isolated T or B cells with or without antigen. However, when these cells were cocultured in the presence of 10 ng/ml streptoccal vaccine, antigen-specific antibody was produced after 8 days of culture and was continuously produced until the experiment was terminated at day 12. Using these combined methods of cell separation and measurement of specific antibody production, experiments are currently in progress to elucidate the function (e.g. help or suppression) of previously established Group C-specific rabbit T cell clones and lines, and to correlate function with phenotypic delineation of rabbit T and B lymphocytes using a panel of monoclonal antibodies previously reported in our laboratory.

D-5

S.KOSIŃSKI and H.IWATEJ

Institute of Immunology and Experimental Therapy, Wrocław, Poland

IN VIVO DETECTION OF TISSUE SPECIFIC ANTIGENS AND SERUM ALLOTYPES IN RABBITS BY A SKIN TEST

In 1970, Hilgrom et al. developed a skin test enabling to detect skin reacting transplantation antibodies /STRA/ in rats. This skin test consists in intracutaneous injection of a post-transplantation serum into an allogeneic rat followed by intravenous injection of Evans blue dye; positive reaction appears as a blue ring. In our work this skin test was used in a search for rabbit STRA, and for antigens, eliciting these antibodies. STRA were found in sera of rabbits immunized with skin allografts. Two specificities designated SA and SB were identified when tested the sera against a panel of rabbits. The SA and SB antigens were different from rabbit histocompatibility system RLA.
The other group of experiments was performed with rabbit antiallotype sera. The skin test with the antiallotype serum was found to give a positive reaction when applied to an animal possessing the corresponding allotype. The allotype skin test was subsequently used for identification of several rabbit serum allotypes; a high degree of correlation of the test with the results obtained with precipitin test was found. The skin lesions generated by this procedure were shown to result from the formation of insoluble immunocomplexes of the antiallotype serum injected and circulating IgG; neither homocytotropic immunoglobulins fixed to cells nor surface immunoglobulins of B lymphocytes were found to take part in this reaction.

D-6

CELL MARKERS

Yun-yi Liu, Chi-tao Chou, S. Dubiski and B. Cinader, Institute of Immunology, University of Toronto.

We have previously identified rabbit T and B cells by two polyspecific antisera, RTLA (Rabbit T Lymphocyte Antigen) and RABELA (Rabbit Bursal Equivalent Antigen). We have now generated two hybridomas which produce antibodies directed against T cells (T_D,1 and T_D,2) and four hybridomas directed against B cells (B_1^S, B_2^S, B_1^A and B_2^A). Monoclonal antibodies, directed against T cells, affect the same group of thymus derived cells as are found to be RTLA-positive. However, the detected antigen is not identical with RTLA since, unlike antisera against RTLA, both monoclonal antibodies react with polymorphonuclear leukocytes. It is, therefore, reasonable to assume that there are two different T cell antigens which are detected by RTLA and the monoclonal antibodies, respectively. The four monoclonal antibodies against rabbit B cells combine with RABELA-positive cells. In addition they combine with a population of spleen cells which is non-T and RABELA-negative. It is intriguing that in the appendix, cells which are RABELA-positive and Ig-negative are reactive with all four of our monoclonal antibodies.

D-7

THE ROLE OF RABBIT IA MOLECULES IN IMMUNE FUNCTIONS AS DETERMINED WITH THE USE OF AN ANTI-RABBIT IA MONOCLONAL ANTIBODY.

STEVEN LOBEL AND KATHERINE KNIGHT. DEPT. OF MICROBIOLOGY AND IMMUNOLOGY, UNIVERSITY OF ILLINOIS AT CHICAGO-HEALTH SCIENCES CENTER, CHICAGO, IL.

We have recently isolated a mouse anti-rabbit Ia monoclonal antibody (MAb) that detects an isotypic determinant present on all rabbit Ia molecules. This anti-Ia MAb inhibits the mixed lymphocyte culture response 30 to 60%. In secondary in vitro immune response cultures this anti-Ia MAb inhibits the proliferative response to poly(GLU50TYR50) and bovine insulin, but does not inhibit the response to KLH or PPD. In studies on mitogenesis it was found that this anti-Ia MAb inhibited the response to LPS but not to Con-A or PHA. The effect of the anti-Ia MAb on other mitogens was found to vary from rabbit to rabbit. In cytotoxicity experiments this anti-Ia MAb kills 35 to 60% of splenic lymphocytes. This treatment decreases the response to B-cell mitogens 40 to 60%, but does not affect the response to T-cell mitogens.

D-8

HETEROGENITY OF RABBIT LYMPHOCYTE POPULATIONS AS DETERMINED BY ULTRASTRUCTURAL LOCALIZATION AND MODULATION OF LYMPHOCYTE SURFACE RECEPTORS FOR MITOGENIC LECTINS (CON A AND PHA) AND ANTI-Ig by Corey Raffel and Stewart Sell. UCSD, San Diego and UT, Houston.

Distribution and modulation of rabbit lymphocyte receptors for anti-Ig, Concanavalin A (Con A), and Phytohemagglutinin (PHA) are examined by electron microscopy and related to lymphocyte activation (blastogenesis). All rabbit lymphocytes have receptors for PHA and Con A but only 70% of peripheral blood lymphocytes (PBL) and 50% of spleen lymphocytes bind anti-Ig. Endocytosis of surface receptors correlates clearly with activation of lymphocytes to blast transformation. By double labeling, about half of the PBL that are activated by Con A or PHA also bear surface Ig. Such cells are found in spleen or lymph nodes in very small numbers. By evaluation of sequential endocytosis, individual rabbit lymphocytes that endocytose only Con A, only PHA, both Con A and PHA, or neither lectin, have been seen. These studies illustrate the heterogenity of lymphocyte populations to mitogen activation and the presence of lymphocytes in rabbit blood that share "T" and "B" cell properties.

Supported by NIH grant CA-16367.

D-9

DISTRIBUTION OF T AND B CELLS IN RABBIT LYMPHOID ORGANS by S. Sell, C. Raffel, and C.B. Scott. UCSD, San Diego and UT, Houston

T and B cell domains are identified in rabbit thymus, lymph node, spleen, appendix, Peyer patch, sacculus rotundus, BALT, and brain by immunofluorescence using anti-thymocyte and anti-immunoglobulin G, A, M and E made in goats. Cortical brain cells label with ATS but not anti Ig. The thymus contains essentially only T cells whereas other organs contain clearly defined T and B cell zones.

The lymph nodes of normal rabbits show little germinal center activity and are mainly composed of diffuse cortex containing T cells with focal B cells in poorly developed primary follicles. The spleen has diffuse T cells in the white pulp with B cell labeling in follicles. Little or no IgA or IgE labeling is seen. In the appendix at least seven distinct zones may be seen with T cells and B cells of different Ig classes located in different areas. The sacculus rotundus and Peyer patches have distribution similar to the appendix. We conclude from these observations as well as studies on proliferation and distribution of lymphoid cells in the rabbit that the GI associated lymphoid tissue is the major source of newly generated T and B cells in the adult rabbit.

see: Devel. Comp. Immunol. 4:355-366, 1980.

D-10

Characterization of two distinct cDNA clones encoding rabbit Class I MHC molecules. Tykocinski, M.L., Max, E.E., and Kindt, T.J.
Laboratory of Immunogenetics, NIAID, NIH, Bethesda, Md.

Amino acid sequence and two dimensional gel electrophoresis data from our laboratory together suggest that there is a restricted repertoire of expressed Class I MHC molecules in the rabbit as compared to man and mouse. To examine rabbit Class I (RLA) expression at the nucleic acid level, we constructed a cDNA library using the Okayama/Berg cloning technique from size-selected poly A mRNA derived from the rabbit T cell line RL5. Thirty-eight clones hybridizing to a murine Class I cDNA probe were isolated, and two representative clones were sequenced. The first clone, pR9, contains a 1363 base pair insert which includes a segment whose translated sequence corresponds precisely to the previously reported amino terminal amino acid sequence for the single RLA molecule detected in RL5 cells. The pR9 insert comprises the complete coding sequence including leader peptide, as well as 5' and 3' untranslated regions. pR9 displays striking sequence homology to HLA. The second clone, pR26, possesses a 1168 base pair insert which comprises most of the coding region and the entire 3' untranslated region of a Class I molecule which is distinct from pR9 and has not been detected in RL5 cells. Intra- and interspecies sequence comparisons point to a distinct clustering of variability.

D-11

FUNCTIONAL STUDIES OF A T CELL SUBPOPULATION IDENTIFIED BY A MONOCLONAL ANTIBODY. James R. Watkins and Katherine L. Knight, Department of Microbiology & Immunology, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

A panel of monoclonal antibodies (Mab) reactive against T cells (9AE10) and T cell subsets (8AC8, 9B4, 1AH10, etc.) have been generated. Mab 8AC8 recognizes a subpopulation of T cells in the thymus and peripheral lymphoid organs. Two color immunofluorescence shows that 8AC8 Mab reacts with a subset of 9AE10 bearing T cells in spleen, thymus, and mesenteric lymph node. Immunofluorescent screening of various tissues with 8AC8 and 9AE10 Mabs revealed that 8AC8 Mab detects approximately 35% of $9AE10^+$ thymus cells and a major subpopulation (from 50 to 80%) of T cells in the peripheral lymphoid organs. Cells from spleen, thymus, and mesenteric lymph node were reacted with 8AC8 Mab; the $8AC8^+$ and $8AC8^-$ cells were sorted on the cell sorter and tested for their ability to respond in vitro to the T cell mitogens Con A, PHA, and PWM. The results show that the mitogen responsive population resides in the $8AC8^+$ cells for all 3 organs tested. Similar results were obtained with $8AC8^+$ and $8AC8^-$ T cells from spleen and MLN; that is, the mitogen responsive population resided in the $8AC8^+$ T cell population and not in the $8AC8^-$ T cell subpopulation. The lack of response of the $8AC8^-$ T cells is thus not due to the presence of B cells in the $8AC8^-$ cells.

To further characterize the $8AC8^+$ and the $8AC8^-$ subpopulations, purified cells were examined for their responses to KLH and their responses in the MLR. Lymph node cells from a KLH immunized rabbit were sorted into $8AC8^+$ and $8AC8^-$ subpopulations and cultured for 5 days in vitro with various concentrations of KLH. The results show that the antigen responsive population is contained within the $8AC8^+$ subpopulation. In MLR experiments, purified $8AC8^+$ cells responded strongly to both allogeneic (SI-242) as well as autologous (SI-64) stimulator cells. In contrast the $8AC8^-$ cells responded much less to allogeneic (SI -18.0) and autologous (SI -4.2) stimulator cells.

Thus, we have separated T lymphocytes into two subpopulations; the $8AC8^+$ and $8AC8^-$ T lymphocytes. The mitogen responsive, antigen responsive and MLR responsive cells reside in the $8AC8^+$ T cell subpopulation.

D-12

SEPARATION OF RABBIT THYMOCYTES AT DIFFERENT STAGES OF MATURATION BY CENTRIFUGATION ON DISCONTINUOUS GRADIENTS OF PERCOLL. James R. Watkins and Katherine L. Knight, Department of Microbiology & Immunology, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

Rabbit thymocytes were separated on discontinuous gradients of colloidal silica (Percoll) into 5 fractions. The fractions were reacted with the monoclonal antibodies 9AE10 and 8AC8, which identify all T cells (9AE10) or a sub-population of T cells (8AC8). The cells were then reacted with FITC goat anti-mouse Ig and analyzed on a Coulter Epics 5 Cell Sorter. The fluorescence profiles revealed that the cells from the thymus fractions differ in their level of expression of antigens detected by the monoclonal antibodies. These differences in the level of antigen expression presumably reflect different stages of T cell differentiation.

The response of Percoll fractionated thymocytes to the T cell mitogens, Con A PHA, and PWM was localized to three of the five fractions. Fractions 2 and 4 responded comparably to the unfractionated thymocytes whereas the response of Fraction 3 was 10-30 times greater than the other fractions or whole thymus. Fractions 1 and 5 did not respond significantly to any of the mitogens. Cells from the five fractions were labeled with ^{111}Indium and injected i.v. to determine the migration patterns of the fractions in vivo. Twenty-four hours later, the various tissues were removed and the amount of radioactivity per gram of tissue was determined for each tissue. The fractions were found to differ in their migration patterns to the various tissues in vivo.

Thus, rabbit thymocytes can be separated into at least five subpopulations which differ in their density, and in the level of expression of T cell antigens, their responsiveness to T cell mitogens and in their in vivo homing characteristics.

D-13

RESTRICTED CHIMERISM IN HISTOCOMPATIBLE RABBIT CELL TRANSFERS. Louise T. Adler, Michelle LeBeau, and Louis J. Annaratone, III. St. Jude Children's Research Hospital, Memphis, TN 38101, and University of Chicago, IL 60637.

Transfer of lymphoid cells (spleen, lymph node, bone marrow, or thymus) from adult donors to RLA-matched newborn rabbits regularly leads to the establishment of lymphocyte chimerism in the recipients. Deliberate mismatching of donors and recipients for *a* and *b* group allotypes showed the continued synthesis of Ig by donor B cells as assessed by Ig products in serum and on lymphocyte membranes. However, under these conditions, evidence so far indicates that T cell chimerism is not established. This conclusion is based on failure to demonstrate the presence of donor type sex chromosomes in lymphocyte cultures stimulated with Con A, which is specific for T cells of the rabbit. On the other hand, B cells with both donor and recipient type sex chromosomes were readily demonstrable in cultures stimulated with a B cell mitogen.

In contrast, when cell transfer conditions were chosen to favor the induction of fatal GvHD (RLA parental type cells into RLA-heterozygous offspring) the small percentage of recipients which survived appeared to be completely reconstituted with cells of the donor's allotype. In such animals complete T as well as B cell conversion to that of the donor's sex was observed. These results suggest that allogeneic stimulation of the donor's T cells may produce conditions favorable to their engraftment and/or that "biological space" created by the attack on the recipient's lymphoid cell compartment is a decisive factor. [Supported by Grants AI 13159, CA 21765 (CORE), CA 09273 from the National Institutes of Health, and by ALSAC]

E. RABBIT MODELS FOR ALLERGY AND INFECTIOUS DISEASES

E-1

MUCOSAL IMMUNOGENICITY IN THE RABBIT OF E.COLI COLONIZATION FACTOR ANTIGENS (CFAs) STERILIZED BY RADIATION OR FORMALIN. E.C.Boedeker, H.H.Collins, S.L.Berman, C.R.Young, M.M.Levine, H.Shoham. Walter Reed Inst. of Res., Washington, D.C. & Center for Vaccine Development, U. of MD., Baltimore, MD.

Purified preparations of CFAs (pili) from enterotoxigenic E.coli (ETEC) are candidates as oral vaccines against TD since native preparations stimulate a mucosal secretory IgA response which could prevent intestinal colonization by organisms expressing such CFAs. Since CFAs are prepared by harvest from cultures of human ETEC, no viable parent organisms must remain in the final oral vaccine preparations. Filtration sterilization has been impractical because CFAs are particulate. Other methods of sterilization, such as radiation or formalin, might alter antigenicity. To compare the immunogenicity of CFA preparations sterilized by gamma radiation (100 kilorads) and formalin (0.1%), we harvested CFA/II pili from an O6:H16 ETEC strain of E.coli (M424C1), documented purity by electrophoresis, sterilized them by radiation or formalin and compared their abilites to stimulate specifc anti-CFA/II IgA following inoculation into rabbit ileal (Thiry-Vella) loops. Loops were inoculated with 8, 1mg doses of radiated or formalin treated CFA/II preparations over 4 weeks. Loop secretions were collected daily and specific anti-CFA/II IgA measured with a double sandwich ELISA with native CFA/II immobilized on the microtiter wells. Formalin and radiation sterilized preparations gave parallel responses at a 1/20 dilution of loop secretions and reached peak and plateau responses during the week following the final immunization. 3 of 4 rabbits immunized with the formalin treated CFA/II preparation had peak and plateau responses indistinguishable from each other and from the responses of rabbits inoculated with radiation-sterilized pili. Mean plateau responses in the two groups were indistinguishable. These results indicate that preparations of CFAs from ETEC can be sterilized either by radiation or formalin, yet retain their mucosal immunogenicity, inducing an IgA response which recognizes native CFA. These results support development of CFA preparations as oral vaccines for TD.

E-2

CHARACTERIZATION, SEPARATION, AND IN VITRO IMMUNIZATION AND CHALLENGE WITH SYNTHETIC PEPTIDES OF RABBIT ILEAL LAMINA PROPRIA MONONUCLEAR CELLS. C.A. Hooper, R.H. Reid, W.T. McCarthy, D.R. Edwards, and M.J. Roy. Depts. of Gastroenterology and Experimental Pathology, Walter Reed Army Institute of Research, Washington, D.C. 20012.

Intestinal intraepithelial and lamina propria lymphoid cells are the first lymphoid cells encountered by intestinally absorbed antigens. In addition, these lymphoid cells reside in the tissue which surrounds gastrointestinal carcinomas. In the immunization experiments, the N-terminal decapeptide of the tridecapeptide used for in vitro cell culture immunization is an antigen on human colonic and stomach carcinomas. The cells used in the cell culture assays were mononuclear cells (MNC) digested from rabbit ileum by collagenase and were separated into two size populations by elutriation. According to examination under the microscope, Coulter Counter sizing, and electron microscopy, the first cell population contained 80% MNC in the $5u^3$ to $30u^3$ cell volume range. 80% of the cells in the second population were MNC in the $180u^3$ cell volume region. Characterization of the small cells showed essentially two cell types. 60% of these cells have lymphocyte morphology, are esterase negative, and do not phagocytose latex particles. 4% of these lymphocytes have surface immunoglobulins characteristic of B lymphocytes, and 35% bind with the anti-rabbit T lymphocyte monoclonal antibody 9AE10 (provided by K.L. Knight, Univ. of Ill.). The remaining 40% of the small cell population have an irregular cell membrane, are esterase positive, and phagocytose latex particles. Of the cell populations, only the population of small size cells responded in the in vitro immunization/challenge assay. The assay measured tritiated thymidine incorporation by cells which had been immunized in culture for 7 days with the tridecapeptide and challenged for 3 days with either the tridecapeptide, the N-terminal octapeptide, or the N-terminal heptapeptide. A series of immunizing concentrations and challenge concentrations were used for each rabbit. These cell characteristics and the cellular responses to the synthetic peptides provide evidence that T lymphocytes and macrophage type cells reside in the lamina propria of the intestine and are capable of cell-mediated immunization and response.

E-3

BASIC ASPECTS OF FREEZE PRESERVATION OF RABBIT EMBRYOS. Suzanne Jackowski, Biochemistry Department, St. Jude Children's Research Hospital, Memphis, TN 38101

A procedural outline of the techniques involved in the freeze preservation of preimplantation embryos is presented. Emphasis is on practical considerations and basic cryobiological principles are included to help explain the methodology. The data used to illustrate the outline cover the following topics: permeation by cryoprotectant, cooling rate, warming rate, critical temperature range and survival assays. The data are largely obtained from studies using mouse embryos and data concerning rabbit embryos are presented as available. Scheduling of ovulation, isolation of embryos and embryo transfer are discussed. A list of equipment and supplies is also included.

E-4

In Vivo and In Vitro Effects of Muramyl Dipeptide in the Rabbit. William C. Kopp, Ph.D., and Hal B. Richerson, M.D. University of Iowa, Iowa City, IA.

We have utilized established rabbit models of hypersensitivity pneumonitis (HP) to study immunologic inflammatory effector mechanisms in the lung. Acute HP develops in systemically immunized rabbits following aerosol challenge with antigen, but continued challenge longer than 3 weeks results in suppression of disease unless muramyl dipeptide (MDP) is added to the aerosol (Am J Pathol 106:409-420, 1982). MDP appears to prevent desensitization by stimulating T_{DH} effector cells with or without direct effects on macrophages. MDP, the minimal adjuvant-active structure that can substitute for mycobacteria in Freund's complete adjuvant, has previously been studied mainly in mice and guinea pigs. In the present studies, *in vitro* effects of MDP on macrophages were examined including enhanced spreading on plastic surfaces, capillary tube migration inhibition and stimulation of 14C-glucosamine uptake. Peripheral blood and lymph node cells were used to evalute mitogenic effects of MDP. No morphologic or functional changes were attributable to MDP in these cellular assays in our models, although positive effects on guinea pig macrophages were demonstrable, and mitogenicity has been reported in the mouse. Results indicate few demonstrable effects of MDP in traditional *in vitro* systems in the rabbit despite remarkable *in vivo* effects, and differences appear to exist among species in responses to MDP *in vitro*. Our model system is potentially employable in the identification of rabbit T cell effector subsets, and in evaluating *in vitro* correlates of cellular hypersensitivity.

E-5

CELLULAR RESPONSES TO HERPES SIMPLEX VIRUS DURING HERPETIC KERATITIS IN THE RABBIT. Roberta H. Meyers-Elliott and Patricia A. Chitjian. Jules Stein Eye Institute, UCLA School of Medicine, Los Angeles, CA 90024.

We have been studying the cellular response to herpes simplex virus (HSV-1) antigens during ocular infection in the experimental rabbit model. In this study, we have examined the nature of the cytotoxic lymphocyte response since cell-mediated immune responses are believed to play a major role in recovery from HSV infections. Three types of lymphocytes have been implicated in destruction of HSV-infected cells: natural killer (NK) lymphocytes, immune killer lymphocytes, and K-cells in antibody-dependent cellular cytotoxicity. We infected New Zealand rabbits by topical instillation of HSV-1 on the abraded cornea. Lymphocytes and serum were obtained at days 3,5,7,12,15 and 20 after infection. Preauricular lymph node cells, peripheral blood lymphocytes, and spleen cells were used as effector cells in a ^{51}Cr-release cytotoxicity assay against HSV-infected and uninfected rabbit corneal cells (RCC). NK activity toward both the immune lymphocyte-mediated cytotoxicity was found at days 7 and 15 after infection. Addition of syngeneic serum to the system blocked effector lymphocyte function at day 15. Cytotoxic responses to HSV-infected RCC were greater than the responses to RCC alone on day 7, but by day 15 the response to RCC was greater, which suggests that some form of "altered-self" antigen may be recognized by the HSV-immune lymphocytes. Immune lymphocytes induced during HSV keratitis were found to protect or inhibit antiviral antibody and complement-mediated cytolysis. Our results suggest that several immune effector mechanisms are involved in recovery from HSV keratitis. HSV-specific immune lymphocytes and antiviral antibody can act alone or in concert with the immune lymphocytes playing the major role.

E-6

Mixed Cryoglobulins And Glomerulonephritis in Schistosoma japonicum Infected Rabbits. A. Robinson, University of Texas Health Science Center, Houston, TX 77025.

Some parasitic infections are associated with immune complex glomerulonephritis (GN). Therefore, S. japonicum infected rabbits were examined for the role that Ag-Ab complexes may play in the development of renal disease. 25 NZW rabbits were infected and followed for 8 months. Periodic renal biopsies revealed changes at six months. Clinical disease was present at 8 months. 5/18 animals had amyloid deposits, 7/18 had mild focal GN, and 5/18 had diffuse, crescentic GN. PAS stain showed wire loops and tubular casts. Immunofluorescence and electron microscopy showed 15/18 animals with diffuse mesangial and peripheral capillary wall IgG deposits; 13/18 with IgM; and 3/18 with C3. Cryoprecipitates (Cryo) of serum are throught to represent Ag-Ab complexes in serum. Cryos were present from 8 weeks on. Immunochemical analysis of Cryo by double diffusion showed IgM and IgG but no parasite Ag. Cryos run on SDS gels demonstrated μ, γ, and light chains. Guinea pigs immunized with purified Cryos in CFA produced Ab to IgM, IgG, and trace amounts of C3, fibrinogen, and α - macroglobulin but no Ab to parasite Ag. Therefore, rabbits with chronic S. japonicum infections have severe renal disease without parasite Ag in the kidneys or Cryos. A possible cause of the renal pathology may be an IgM anti-IgG rheumatoid-factor-like antibody induced in response to chronic infection.

E-7

HISTOPATHOLOGY AND IMMUNOPATHOLOGY OF EXPERIMENTAL SYPHILIS IN THE RABBIT by S. Sell, S. Lukehart, S. Baker-Zander, and S. Norris, UCSD, San Diego, UT Houston

Experimental intradermal or intratesticular infection of rabbits with T. pallidum initiates a prompt systemic hyperplasia of lymphoid organs, the appearance in lymphoid organs of T lymphocytes responsive to T. pallidum antigens, rising titers of humoral antibody to T. pallidum, and extensive local monoclonal inflammatory reaction. An early (5-9 days) lymphocyte (T cell) infiltrate is replaced by macrophages which phagocytose and digest T. pallidum in situ. The evolution of the inflammatory response is essentially that of a primary delayed hypersensitivity reaction.

Complete concommitant immunity to T. pallidum occurs three months after primary infection and has not been induced by non-infectious immunization. In the skin of chancre immune rabbits a mononuclear perivascular reaction peaks at 4-5 days with little or no gross evidence of inflammation. There appears to be selective localization of T. pallidum in cutaneous nerves and erector pili muscles. The mechanism of organism elimination in immune animals is under active investigation.

Tentatively, we conclude that macrophage destruction of T. pallidum as a consequence of a delayed hypersensitivity reaction is the major mechanism for removing organisms from the site of infection. Although a role of humoral antibody cannot be demonstrated, it could be responsible for resistance to infection by other immune mechanisms. Latency is most likely the result of the localization of organisms in tissues that are resistant to immune inflammation.

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E-8

IMMUNOBIOLOGICAL FUNCTIONS OF RABBIT SYNOVIAL CELLS. Moti L. Tiku, Marius Teodorescu and John L. Skosey, University of Illinois College of Medicine, Chicago, IL 60612, USA

Rabbits have served as convenient and widely used animals for production of experimental arthritis. Antigen induced arthritis consists of injection of antigen material in the joint cavity. The cells responsible for initiation and perpetuation of joint pathology remains unknown. We therefore assessed the ability of enzyme-dissociated primary cultures of synovial cells (SyC) to present antigen and to induce allogenic or autologous mixed lymphocyte reaction (MLR or AMLR). Adult rabbits were immunized in foot pads with bovine serum albumin (BSA) in complete Freund's adjuvant (CFA) or CFA alone. Four to six weeks later draining popliteal lymph node cells (LNC) and synovial cells were obtained. Synovial cells were cultured overnight with or without antigen. As a positive control splenic adherent cells (SAC) were similarly treated. Next day, to the extensively washed and irradiated synovial cells or splenic adherent cells, autologous lymph node cells were added. Lymphocyte proliferation was assessed by 3H-thymidine uptake. We found that synovial cells as well as splenic adherent cells (Table) induced MLR and AMLR and effectively presented antigen for specific immune response to the priming antigen. About 20% of synovial cells had Fc receptors and 15% C_3 receptors. These studies demonstrate that normal rabbit synovium contains cells which can present antigen, and are able to induce MLR & AMLR. Presence of such cells in synovium may be responsible for initiating and sustaining immune reactivity in immological mediated arthritis.

CELLS	CPM ± SD
SyC	646 ± 82
SyC+LNC	24586 ± 3163
BSA-pulsed SyC+LNC	31434 ± 3819
SAC	239 ± 41
SAC+LNC	


FILME
1-84